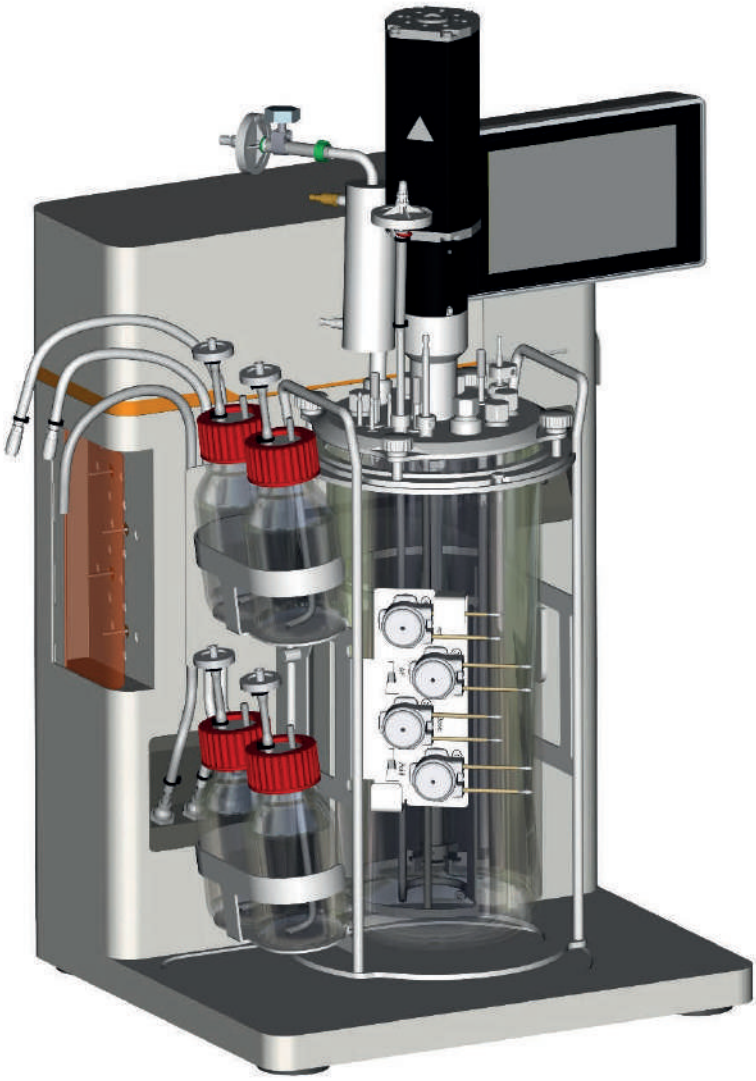
Operating Manual

Minifors 2

Benchtop Bioreactor



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Engineering and production in Switzerland

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1 General Information

1.1 About this Manual

This manual enables the safe and efficient handling of the equipment.

All the information and instructions in this operating manual comply with the current standards, legal regulations, the latest technological and scientific developments and the knowledge gained from the manufacturer's many years of experience in this field.

This operating manual is a component part of the equipment. It must be kept near to the equipment and must be accessible to the operators at all times.

The users must read the operating manual thoroughly and fully understand its contents before beginning any work.

Adhering to all the safety and operating instructions in this manual is essential to ensure that work is carried out safely.

The scope of delivery may differ from the explanations, descriptions and figures in this operating manual due to special designs, additional options specified on ordering and the latest technical/mechanical modifications.

This manual contains illustrations to aid general understanding. These may differ from the actual equipment as supplied.

1.2 Explanation of Special Notices

1.2.1 Warning Notices

Warning notices in this manual are indicated by a coloured bar and begin with a signal word that signifies the degree of the hazard.

DANGER

The signal word "DANGER" indicates a dangerous situation that will lead to severe or even fatal injuries if not avoided.

WARNING

The signal word “WARNING” indicates a potentially dangerous situation that may result in severe or even fatal injuries if not avoided.

CAUTION

The signal word “CAUTION” indicates a potentially dangerous situation that may result in minor injuries if not avoided.

1.2.2 Other Notices

ATTENTION

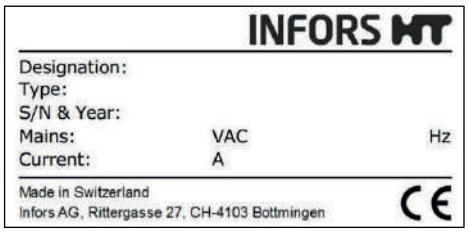
The word “ATTENTION” on a blue bar indicates a situation that may result in significant damage to property if not avoided.

INFORMATION

Texts located below a grey bar bearing the notice “INFORMATION” provide useful tips and recommendations for ensuring efficient, fault-free operation of the equipment.

1.3 Equipment Identification (Standard Identification Plate)

The identification plate is designed to allow clear identification of the equipment. It contains the following information:



- Manufacturer name
- Designation = Category of equipment
- Type = Equipment type (name)
- S/N = Serial number
- Year = Year of manufacture
- Mains = Nominal voltage and frequency
- Current = Current consumption
- Manufacturer address
- CE marking

1.4 Declaration of Conformity

The equipment is in compliance with the essential requirements of the following Directives:

- Machinery Directive 2006/42/EC
- EMC Directive 2014/30/EU

The Declaration of Conformity according to EC Machinery Directive 2006/42/EC, annex II 1 A is included in the general documentation supplied with the equipment.

1.5 Customer Service and Services

Our Customer Service is at your disposal for technical advice and specialist enquiries. For contact information, see page 2.

Due to their familiarity with the potential applications of the equipment, the Customer Service team is able to provide information on whether the equipment can be used for a specific application or modified to handle the planned process.

Experience of working with the equipment will be published semi-regularly on the manufacturer's website in the form of "application notes".

Furthermore, our colleagues are always interested in new information and experiences resulting from user's applications for the equipment that may be valuable for the continued development of our products.

2 Safety and Responsibility

This section describes general considerations relating to user safety that must be taken into account when working with the equipment.

In the remaining sections, warning notices are used only to highlight particular hazards directly arising from the actions being described in the section in question.

It is essential to read the operating manual carefully – especially this section and the warning notices in the text – and to follow the instructions therein.

This section also refers to areas that are the responsibility of the provider due to certain risks arising from particular applications for which the equipment is used deliberately and with full awareness of the associated risks.

2.1 Intended Use, Incorrect Use and Misuse

The bench-top bioreactor Minifors 2 from INFORS HT is designed especially for research and development and for the cultivation of microorganisms in a biotechnology laboratory.

The equipment is designed and constructed exclusively for the intended use described above.

Intended use also includes following all the instructions in this operating manual, especially those relating to:

- The installation site
- User qualifications
- Correct operation and maintenance
- The use of undamaged tubing and glass vessels

Any failure to observe the requirements specified in this manual shall be deemed incorrect use.

Any use of the equipment outside the scope of the intended use as described above shall be deemed misuse.

This also applies to applications for which the equipment is not designed, such as the use or production of explosive gases, which is not permitted because the equipment is not explosion-proof.

For use for special applications not covered by conventional, intended use, the equipment must be modified and certified accordingly by the manufacturer.

Any use of the equipment outside of a biotechnology laboratory, i.e. in any environment in which the conditions required for the safety of the users cannot be fulfilled or cannot be fulfilled to their full extent, shall also be deemed misuse.

2.2 Qualified Personnel

Due to the complexity of the equipment and the potential risks arising from its operation, the equipment may only be used by qualified, specialist personnel.

2.2.1 Provider

The term “provider” applies to all persons who are responsible for making the equipment and the necessary infrastructure available. These persons may also be included in the group of people known as “users”, though this is not always the case.

Irrespective of whether a provider is a member of the company’s board of management or a supervisor, they bear a special level of responsibility with regard to the processes and the qualification and safety of the users.

2.2.2 User

General

The term “user” applies to all persons who come into contact with the equipment in any way and perform work on or with it. This primarily applies to the following activities, which can be performed by the manufacturer’s own specialists or a variety of other persons (it is not always possible to distinguish clearly between the different types of person):

- Assembly, installation and commissioning
- Definition and preparation of the process
- Operation
- Troubleshooting and remedying of faults
- Maintenance and cleaning (autoclaving, if necessary)
- Service work and repairs
- Disassembly, disposal and recycling

Qualified personnel

On account of their specific education, training and – in many cases – experience, the qualified personnel required for this work are able to recognise risks and respond accordingly to potential hazards.

The qualified personnel (either internal or external) who cannot be categorised under the separate “operators” group are made up of the following groups of persons:

- Electricians (electrical engineers)
- Decontamination specialists
- Repair specialists
- Specialists in disassembly and (environmentally friendly) disposal
- Recycling specialists

2.2.3 Operator

The “operators” are a specific sub-group of users distinguished by the fact that they work with the equipment. They are the true target audience for this operating manual.

Qualified technicians

Only technicians who have been trained for working in a biotechnology laboratory can be considered for the role of operator. These include:

- Process technicians in the fields of biotechnology and chemistry
- Biotechnologists (biotechnicians)
- Chemists with a specialisation in biochemistry; chemists in the field of organic chemistry or biochemistry
- Life scientists (biologists) with special education in cytology, bacteriology, molecular biology, genetics, etc.
- Lab assistants (lab technicians) from various fields

In order to be classed as a “sufficiently qualified technician” for the operation of the equipment, the persons in question must have received thorough training and have read and understood the operating manual.

The operator must be informed in a training session provided by the provider of the tasks delegated to the operator and the potential risks of improper conduct. Tasks that go beyond the scope of operation under normal conditions may only be performed by the operator if this is specified in the manual and the provider has explicitly entrusted said tasks to the operator.

Technicians in training

Persons in this group who are undergoing training or apprenticeships are only permitted to use the equipment under supervision and in accordance with the instructions of a trained and qualified technician.

2.3 Unauthorised Persons

The term “unauthorised persons” applies to all persons who can access the work area but are not qualified to use the equipment in accordance with the aforementioned requirements.

Unauthorised persons are not permitted to operate the equipment or use it in any other way.

2.4 Responsibility of the Provider

The equipment is used for industrial and scientific purposes. As such, the provider of the equipment is individually liable with regard to the legal requirements relating to occupational health and safety in a biotechnology laboratory. In particular:

- The provider is responsible for ensuring that the work and environmental regulations applicable in a biotechnology laboratory are observed.
- The provider must ensure that the equipment remains in safe and proper working condition throughout its entire term of use.
- The provider must ensure that all safety equipment is fully functional and is not disabled.
- The provider must ensure that the equipment is only worked on by qualified users, and that said users receive sufficient training.
- The provider must ensure that the protective equipment required for working with the equipment is provided and worn.
- The provider must ensure that this operating manual remains in the immediate vicinity of the equipment throughout its entire term of use.

2.5 General Hazards

This section covers general hazards and residual risks that are always present when using the equipment in accordance with normal, intended use.

The following notices are general in nature. As such, with a few exceptions they are not repeated in the remaining sections.

2.5.1 Electrical Current

The equipment runs on electrical power. There is an immediate risk of fatal injury if contact is made with live parts.

The following points must be observed in order to avoid the risk of fatal injury:

- In case of damage to insulation, disconnect the equipment from the mains immediately and arrange for it to be repaired.
- Disconnect the equipment from the mains before commencing any work on the electrical system.
- Always use qualified electricians for any work on the electrical system.
- Keep moisture away from live parts. It may lead to a short circuit.

2.5.2 Unauthorised Spare Parts and Accessories

Incorrect or imitated spare parts and accessories as well as spare parts or accessories that have not been authorised by the manufacturer represent a significant safety risk. As such, we recommend procuring all spare parts and accessories from an authorised dealer or directly from the manufacturer. For the contact details of the manufacturer's representatives, see page 2.

2.6 Particular Hazards

This section covers particular hazards and residual risks that may arise when using the equipment for special applications in accordance with normal, intended use.

Since the use of the equipment for such applications is deliberate, it is the responsibility of the operators and the provider to ensure that all personnel are protected from potential damage to health. The provider is responsible for ensuring that the appropriate protective equipment for such applications is provided, and that the necessary infrastructure is in place.

2.6.1 Hot Surfaces

For processes that are carried out with temperatures over 55 °C, there is a danger of burns on hot surfaces.
Since the equipment is intended for applications at high temperatures, it is the responsibility of the users to ensure that they have sufficient protection.

The motor gets hot during operation. There is a risk of burns if it is touched.

The thermal block and its adapter get hot during operation. There is a risk of burns, if touched.

2.6.2 Dangerous Gases

The use or production of dangerous gases i.e. toxic or asphyxiant gases entails a significant health risk, especially in enclosed spaces.

In order to prevent high emissions of dangerous gases, the following measures must be taken:

- The gas connections on the equipment must be checked before any cultivation processes using dangerous gases are initiated.
- The gaskets on the equipment must be checked at regular intervals and replaced if necessary.
- Siphon off exit gas safely.

2.6.3 Flammable or Explosive Substances

The use or production of flammable or explosive substances is not covered under “intended use” of the equipment, as the equipment is not explosion-proof.

If the provider intends to use the equipment for such purposes, he must check its suitability for the planned application with the responsible local authorities.

2.6.4 Corrosive or Toxic Substances

The use or production of corrosive or toxic substances entails a significant health risk. As such, special measures must be taken to protect the users for such applications.

Since the equipment is used deliberately for such applications, it is the responsibility of the users to ensure that they have sufficient protection.

2.6.5 Bioactive Substances or Pathogenic Organisms

The use or production of bioactive substances, pathogenic organisms or genetically modified cultures entails a significant health risk. As such, special measures must be taken to protect the users for such applications.

Since the equipment is used deliberately for such applications, it is the responsibility of the users to ensure that they have sufficient protection.

2.6.6 Overpressure or Vacuum

Glass vessels may break or shatter when subjected to overpressure or vacuums.

2.7 Warning Symbols on the Equipment

The following warning symbols (stickers) are attached to the equipment:

Position

- Thermal block adapter
- Motor

WARNING

Illegible or missing warning symbols on the equipment will lead to the user being exposed to risks that the warning symbols in question were designed to make him or her aware of.

It is the provider's responsibility to ensure that all the stickers with warning symbols on the equipment are always intact.

2.8 Declaration of Decontamination

When returning the equipment for repair, disassembly or disposal, it is required for the safety of all parties involved and because of legal provisions that a lawful declaration of decontamination is present.

The following must be observed if this is the case:

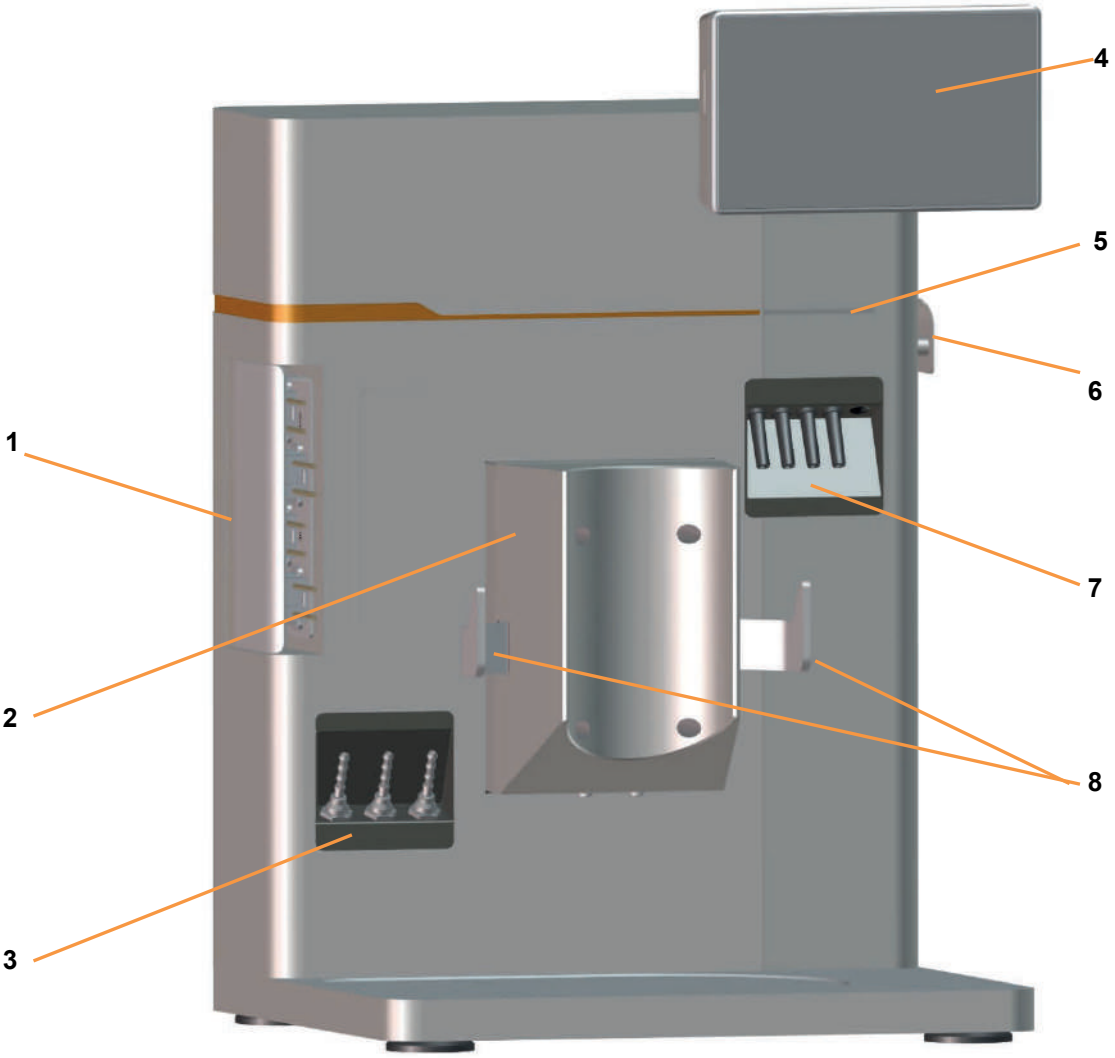
- The equipment, the component part or accessory must be entirely decontaminated before sending to the manufacturer
- The provider is therefore required to completely and truthfully fill out a declaration of decontamination, and have it signed by the person responsible.
- **The declaration of decontamination must be affixed on the outer packaging in which the equipment is sent back.**
- These forms can be obtained from the licensed dealer or the manufacturer. See address on page 2.

Important notice

If the return shipment is not accompanied by a signed and complete declaration of decontamination or it is not affixed to the outer packaging, the shipment will be returned unopened to the sender at their expense (see also T&C).

3 Setup and Function

3.1 Basic Unit



1	Pumps	5	LED signal strip
2	Thermal block and adapter	6	Main switch
3	Connections for sparger and exit gas cooler	7	Connections for sensors
4	Operating panel	8	Hooks for vessel holder

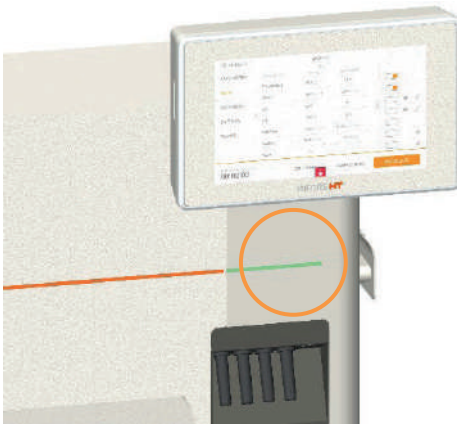
All of the measurement and control technology is built into the basic unit. The basic unit is equipped as standard with a thermal

block plus adapter for regulating the temperature of the culture vessel, four pumps for adding reagents and feed solution, and the operating panel.

3.1.1 Main Switch

The main switch is located on the right-hand side on the basic unit. It lights up green as soon as it is switched on.

3.1.2 LED Strip



The LED strip is located on the front of the basic unit.

When the LED strip:

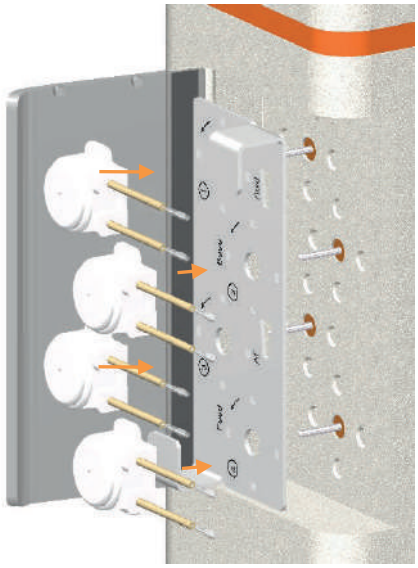
- Lights up green as soon as the equipment is switched on. The equipment is functioning as normal.
- Flashes green. This indicates a parameter alarm (for more details, see the “Operation” section).
- Flashes red. This indicates an Equipment error (for more details, see the “Interferences” section).

3.1.3 Pumps

Reagents and feed solution are supplied via four peristaltic pumps. The pumps are driven by stepper motors.

The pump drive shafts are located on the left-hand side of the basic unit. The drive shafts' direction of rotation is set as standard to anti-clockwise for "filling"; see marking on mounting plate. The pumps can be configured individually using the operating panel, and thus each set to digital or analogue operation as required.

A hinged Plexiglas cover acts as a guard during operation.



The autoclavable pump heads are plugged into a mounting plate. (Depicted separately here in order to show the marking under the pumps.) This is numbered 1 to 4 from bottom to top, and labelled to indicate the standard factory settings:

- Pump 1: *Acid* (digital)
  Alternative setting: FEED (analogue)
- Pump 2: *Base* (digital)
  Alternative setting: FEED (analogue)
- Pump 3: AF (antifoam, digital)
  Alternative setting: LEVEL (digital) or FEED (analogue)
- Pump 4: *Feed* (analogue)
  Alternative setting: BALANCED (analogue)

For more information on possible pump settings, see the "Operation" section, sub-section "Parameter Group PUMPS".

The pump heads and the mounting plate can be simply pushed onto or pulled off the drive shafts.

3.1.4 Identification Plate

The identification plate is located at the side of the basic equipment.

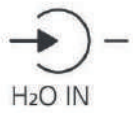
The data provided on the identification plate is specified in the main chapter "General Information", chapter "Equipment Identification".

3.1.5 Power Connection

The connection socket for the power cable is located on the rear of the basic unit, at the bottom left.

3.1.6 Water Connections

The water connections are located on the rear of the basic unit, at the bottom right. They are marked with the following symbols:

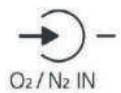


- **H_2O IN**: water inlet

- **H_2O OUT**: water outlet

3.1.7 Gas Connections

The connections for the gas supply are located on the rear of the basic unit, at the bottom right, above the water connections.They are marked with the following symbols:



- Inlet oxygen / nitrogen

- Inlet air

3.1.8 Signal Connections

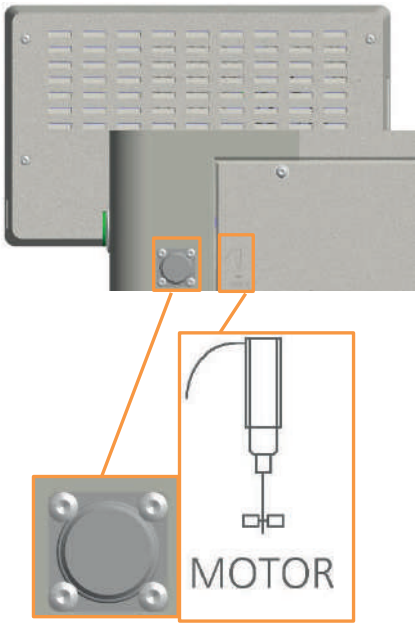
The following signal connections with the corresponding symbols are located on the rear of the basic unit, at the bottom left:

- SERVICE: 9-pin RS232, for connecting a diagnostic computer for maintenance.

- BALANCE: 9-pin RS232, for connecting a balance

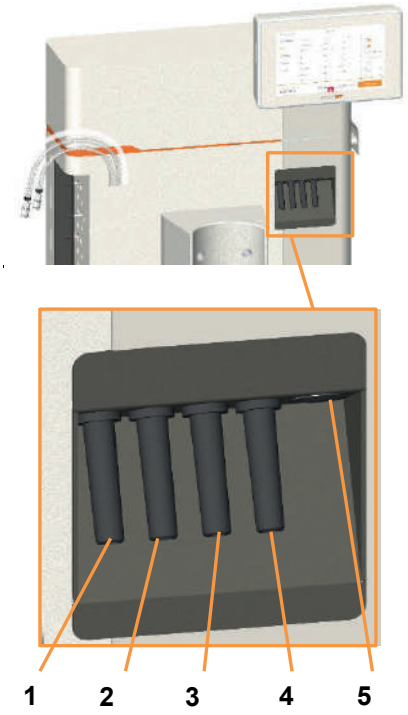
- LAN port: for connecting a network cable

3.1.9 Motor Cable Connection



The connection for the motor cable is located on the rear of the basic unit at the top left, and marked with a corresponding symbol.

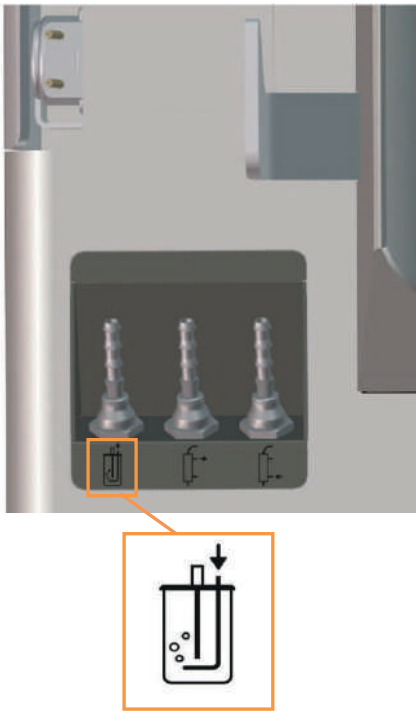
3.1.10 Connections for Sensors (Sensor Cables)



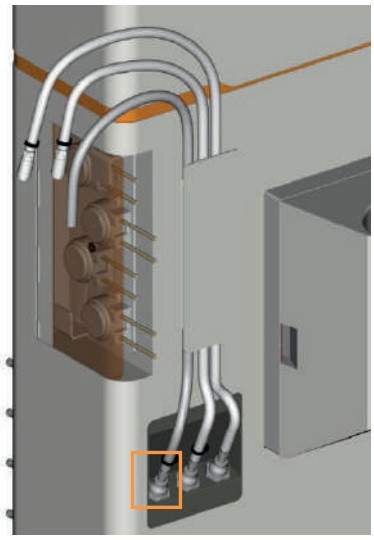
The basic unit is equipped as standard for measuring temperature, pH, pO_2 and antifoam. This means that the temperature (Pt100) and the connecting cables for the pH, pO_2 and antifoam sensors are always present. The matching sensors for pH, pO_2 and antifoam are included in the standard package.

1 pO_2
2 Temperature (Pt100)
3 Antifoam
4 pH
5 Spare connection for turbidity measurement sensor (option)

3.1.11 Sparger Connection (Gassing)

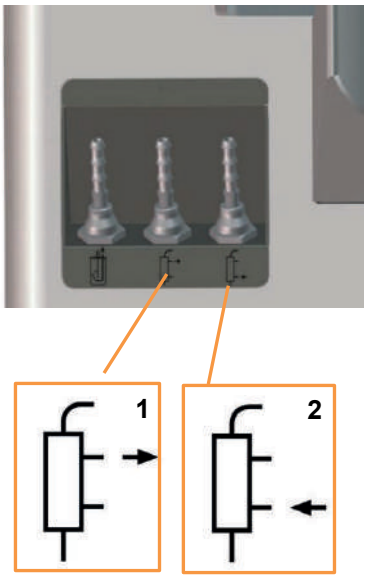


The connection for the gassing (sparger) is located on the front of the basic unit, at the bottom left. It is marked with a corresponding symbol.



The hose used to connect the sparger is connected to the basic unit's gassing connection at the factory.

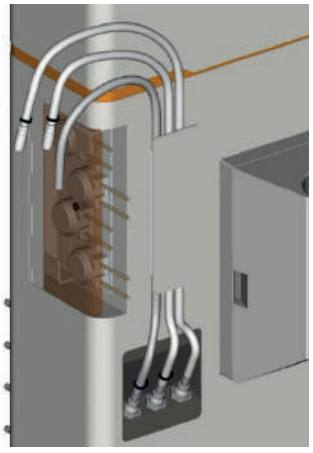
3.1.12 Connections and Water Flow Control Valve for the Exit Gas Cooler



The water connections for the exit gas cooler are located on the front of the basic unit, at the bottom left. They are marked with the corresponding symbols:

1 Exit gas cooler water outlet

2 Exit gas cooler water inlet



The water supply and return hoses for the exit gas cooler are connected to the basic unit in the factory.The rapid couplings at both ends of the hoses are used to connect them to the exit gas cooler.

INFORMATION

Due to the different hose lengths, it is not possible to connect them to the exit gas cooler incorrectly.

The water flow control valve is located on the rear of the basic unit and marked with a corresponding symbol:

The control valve is set at the factory. If necessary, the water flow rate can be set manually using the control valve.

- Turn anti-clockwise to increase the water flow rate
- Turn clockwise to reduce the flow rate

The valve can be fixed in the required position using a lock nut.

3.2 Operating Panel

The operating panel on the top right of the basic unit has a 7” TFT colour touch screen.

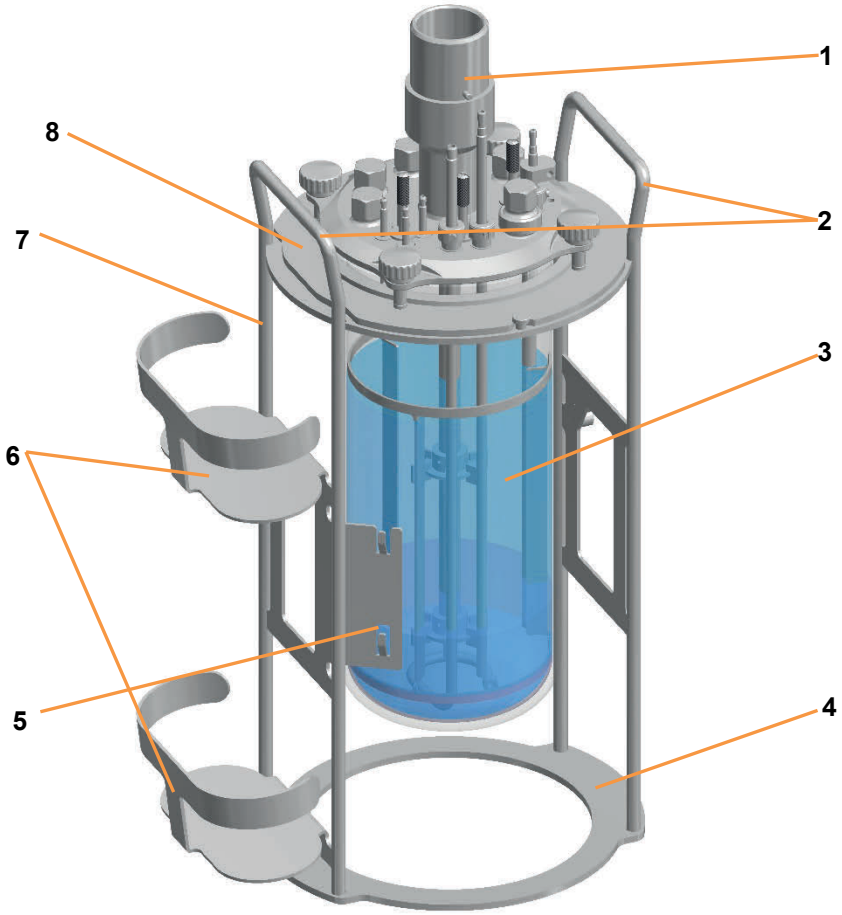
- On the right-hand side of the panel is a USB port.
- On the left-hand side is a slot for an SD card

The operating panel is switched on using the main switch. For a detailed description of how to operate it, see the “Operation” section.

3.3 Culture Vessel

The culture vessel comprises a glass vessel, the top plate with the standard fittings (which vary based on vessel size) and the vessel holder with handles. The vessel is made of borosilicate glass.

Setup and Function

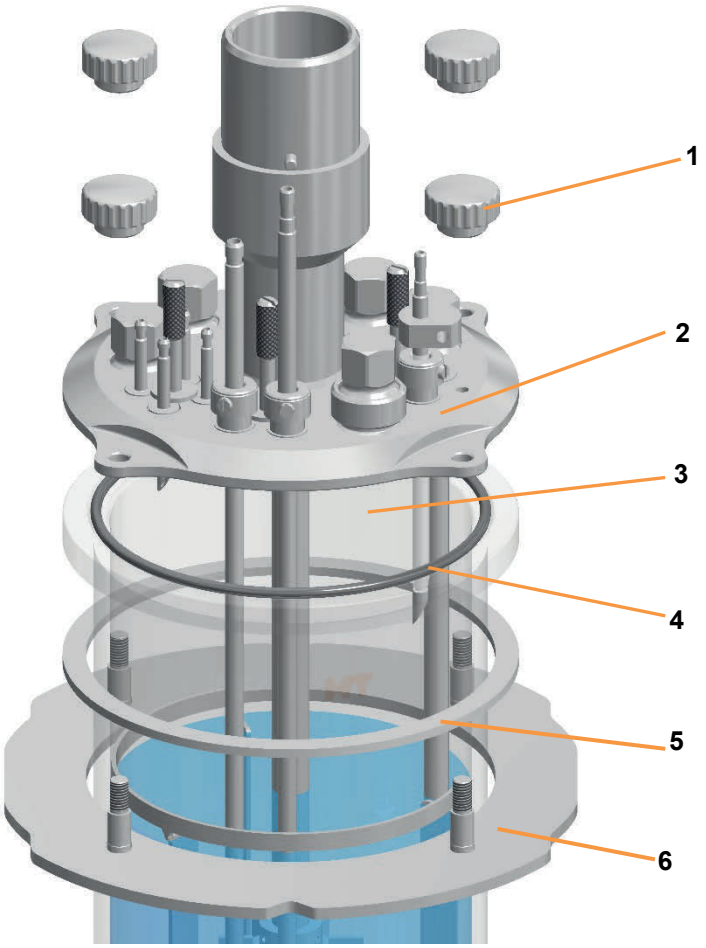


1	Motor coupling	5	Pump holder
2	Vessel holder handle	6	Reagent bottle holder
3	Glass vessel	7	Vessel holder
4	Vessel holder stand	8	Top plate

The illustration shows a culture vessel with a total volume of 1.5 L and a nominal width of 90 mm. There are three vessel sizes available, each with a matching top plate.

The vessel holder has two handles on the side, which are used when emptying and cleaning the vessel or transporting it to the autoclave.

3.3.1 Top Plate



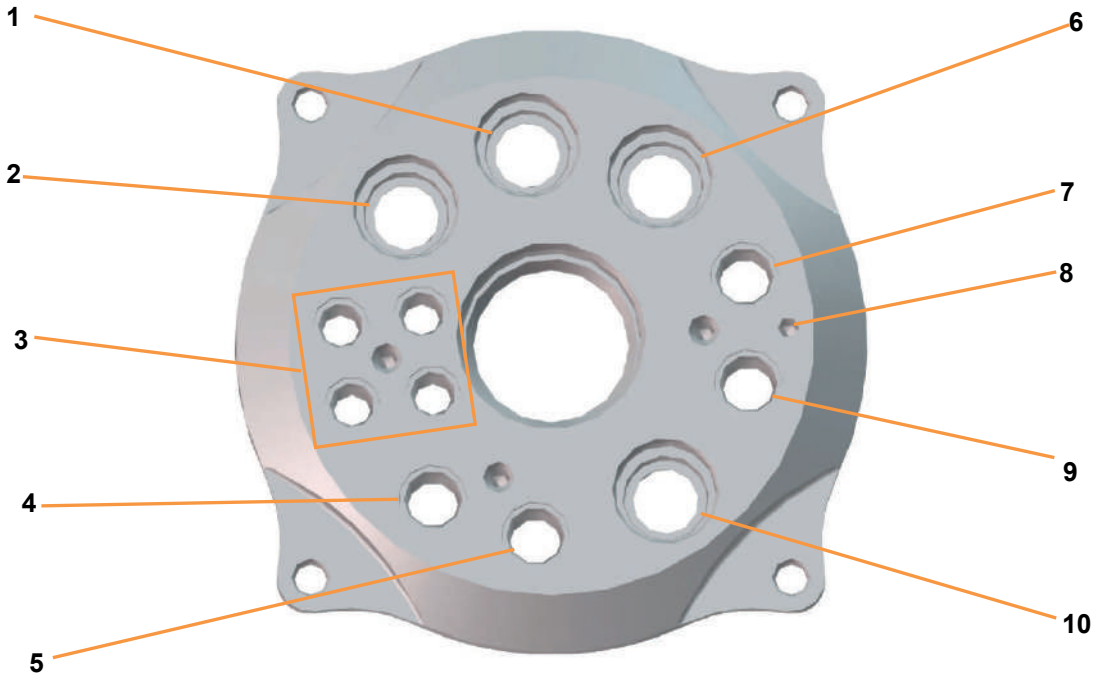
1	Knurled screw (x4)	4	O-ring
2	Top plate	5	Damping ring (spacer ring)
3	Vessel	6	Flange

The top plate is attached to the vessel using four knurled screws and a flange. The knurled screws also hold the vessel in place in the vessel holder. An O-ring is used to seal the top plate. A spacer ring is used to prevent the top plate from exerting pressure on the rim of the vessel.

3.3.2 Ports in the Vessel Top Plate and their Configuration

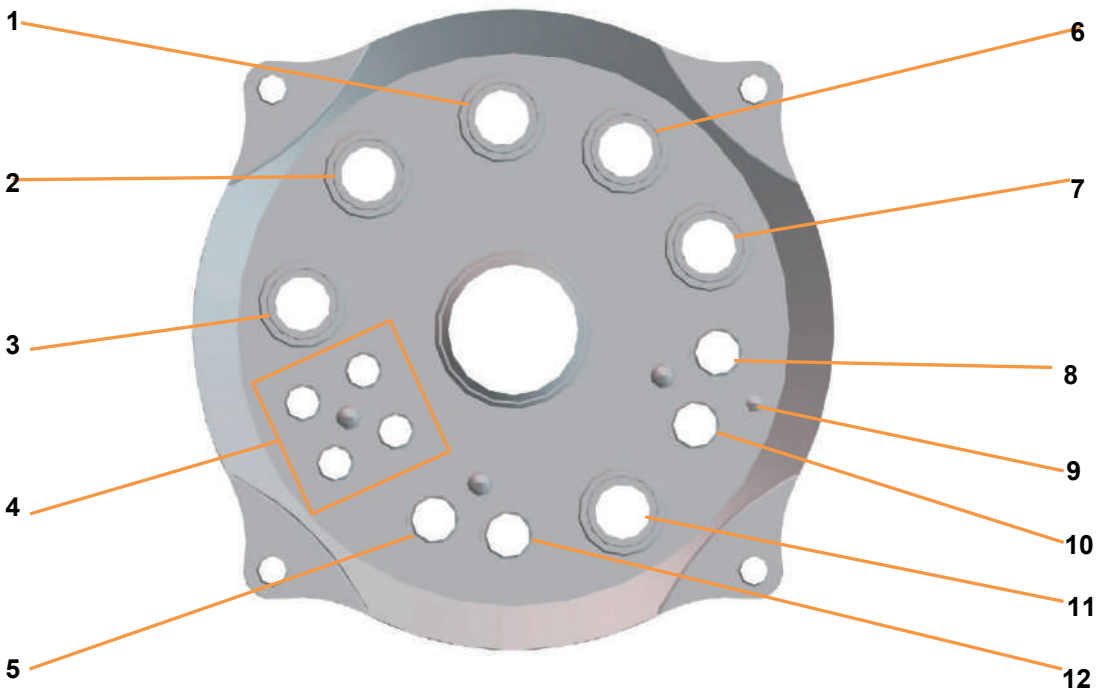
The vessel top plate has different ports of different sizes to mount the different components such as sparger, blanking plugs, sensors etc. The number of ports in the top plate and its configuration depends on the diameter nominal (= inner diameter) of the culture vessel.

3.3.3 Vessel Top Plate, Nominal Width 90



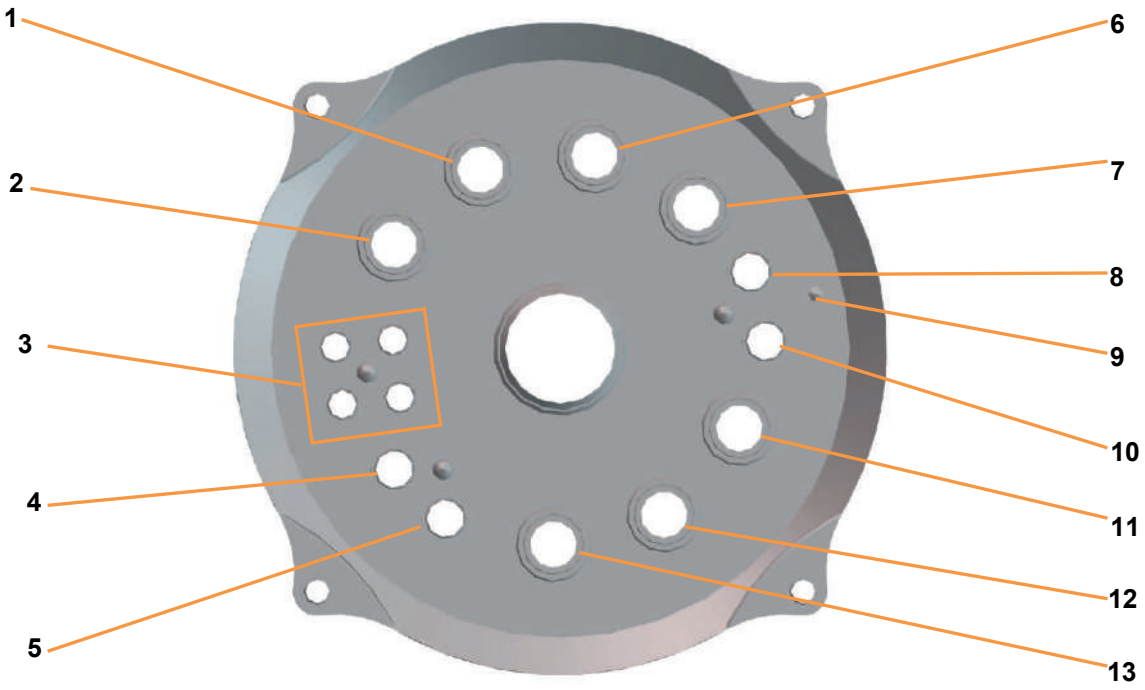
1	Ø 12 mm Pg13.5: pH sensor	6	Ø 12 mm Pg13.5: pO_2 sensor
2	Ø 12 mm Pg13.5: Exit gas cooler	7	Ø 10 mm: Temperature sensor (Pt100)
3	Ø 7.5 mm: Addition port adaptors (4x)	8	Antifoam sensor earth connection
4	Ø 10 mm: Sparger	9	Ø 10 mm: Antifoam sensor
5	Ø 10 mm: Dip tube for sampling	10	Ø 12 mm Pg13: Inoculation

3.3.4 Vessel Top Plate, Nominal Width 115



1	Ø 12 mm Pg13.5: pH sensor	7	Ø 12 mm Pg13.5: Additional sensor
2	Ø 12 mm Pg13.5: Exit gas cooler	8	Ø 10 mm: Temperature sensor (Pt100)
3	Ø 12 mm Pg13.5: Additional sensor	9	Antifoam sensor earth connection
4	Ø 7.5 mm: Addition port adaptors (4x)	10	Ø 10 mm: Antifoam sensor
5	Ø 10 mm: Sparger	11	Ø 12 mm Pg13.5: Inoculation
6	Ø 12 mm Pg13.5: pO_2 sensor	12	Ø 10 mm: Dip tube for sampling

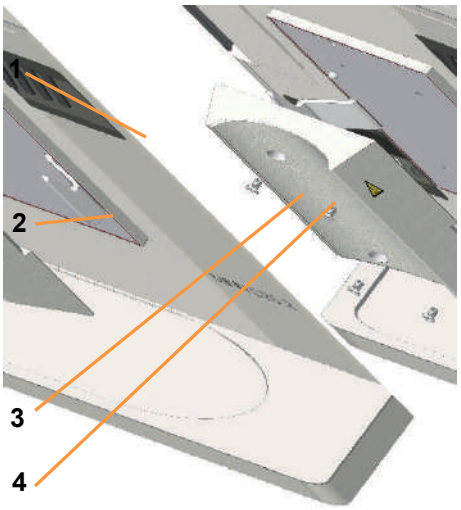
3.3.5 Vessel Top Plate, Nominal Width 145



1	Ø 12 mm Pg13.5: Exit gas cooler
2	Ø 12 mm Pg13.5: Additional sensor
3	Ø 7.5 mm: Addition port adaptors (4x)
4	Ø 10 mm: Sparger
5	Ø 10 mm: Dip tube for sampling
6	Ø 12 mm Pg13.5: pH sensor
7	Ø 12 mm Pg13.5: pO_2 sensor
8	Ø 10 mm: Temperature sensor (Pt100)
9	Antifoam sensor earth connection
10	Ø 10 mm: Antifoam sensor
11	Ø 12 mm Pg13.5: Additional sensor
12	Ø 12 mm Pg13.5: Additional sensor
13	Ø 12 mm Pg13.5: Inoculation

3.4 Temperature Control System

The temperature (heating and cooling) is controlled using a thermal block and its adapter.

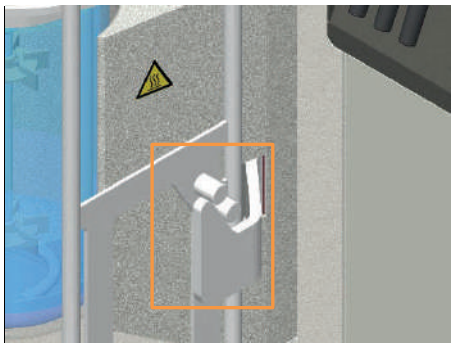


1 Thermal block adapter
2 Thermal block
3 Fastening screw (Allen screw, 4 pieces)
4 Hook, 2 pieces

There is a thermal block adapter for each vessel size. The thermal block adapters are screwed onto the thermal block.

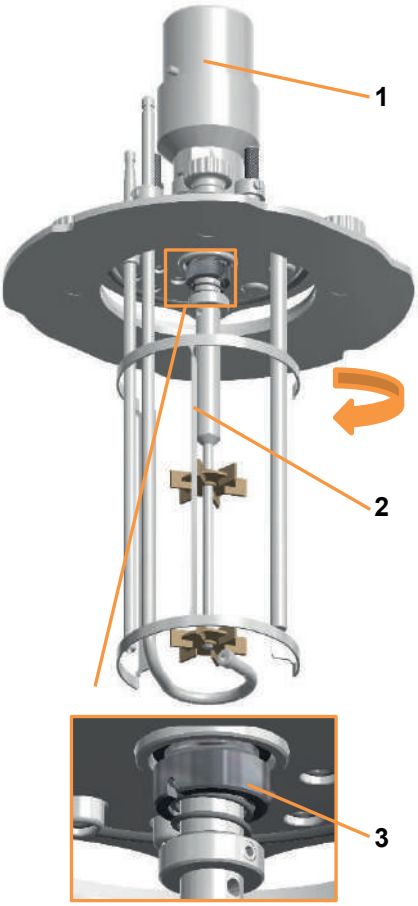
The temperature in the culture vessel is measured using a platinum resistor temperature sensor (Pt100). The temperature is transmitted from the thermal block to the adapter and from the adapter to the culture vessel by means of heat exchange.

The thermal block is heated electrically using heating cartridges. It is cooled by allowing cooling liquid to flow through it.



The two hooks on the thermal block hold the culture vessel in place on the basic unit. In order to ensure optimum heat transmission, the two hooks also pull the culture vessel right up against the thermal block.

3.5 Stirrer



The stirrer shaft is driven from above and turns anti-clockwise (left when viewing vessel from above).

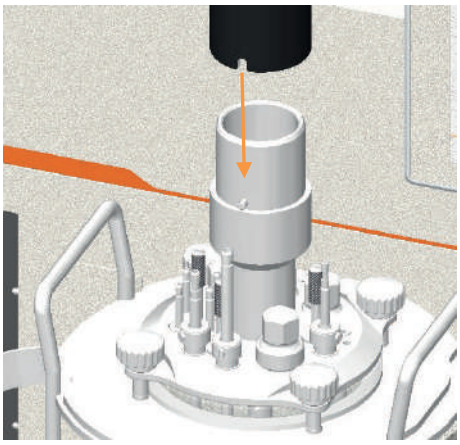
1 Drive hub
2 Stirrer shaft
3 Mechanical seal

The stirrer shaft is sealed using a mechanical seal.

Two Rushton impellers are attached to the stirrer shaft by means of grub screws.

A brushless gear motor with a mechanical coupling is used as standard. Depending on the size of the vessel, two motors with different power levels are used; see the “Technical data” section, sub-section “Stirrer specifications”.

- Left: Small motor for culture vessels with nominal width 90
- Right: Large motor for culture vessels with nominal widths 115 and 145



The motor is connected by pushing it onto the drive hub on the top plate.

3.6 Gassing System

The following gases can be used:

- Air
- Oxygen (O_2) or nitrogen (N_2)

The basic unit is equipped and configured with two mass flow controllers for controlling the gas flow and, if necessary, the gas mixture. If oxygen or nitrogen are used in addition to air, the gases are mixed before being fed into the culture vessel. Both the gas flow rate(s) and the composition of the gas mixture (where applicable) are set using the operating panel. For more details, see the “Operation” section.

3.6.1 Gas Entry

A silicone hose leads the gas or gas mixture from the gassing connection on the basic unit to the culture vessel, via a sterile filter. The gas is fed directly into the medium via the sparger (sparger gassing). For more details on the sparger, see the “Accessories” section, sub-section “Sparger”.

3.6.2 Exit Gas

Even without active gassing, any cultivation can increase the pressure inside the vessel through heating or gas production. As such, an exit gas line is essential for all cultivation processes.

Siphoning off exit gas via the exit gas cooler

The exit gas cooler dries the exit gas through condensation, thus preventing the exit gas filter from becoming clogged with moisture. At the same time, it also prevents liquid loss in the culture medium.

INFORMATION

If heavy foaming is expected, a bottle of antifoam agent can be installed upstream of the exit gas filter as a foam trap.

The exit gas cooler is included in the standard package; for more details, see the “Accessories” section, sub-section “Exit Gas Cooler”.

3.7 pH Control

pH in the medium is measured by the pH sensor and regulated by addition of reagents (acid, base). Addition of acid and base takes place via the two peristaltic pumps *Acid* and *Base* .

Reagent bottles are filled with acid and base which are connected to an addition port adapter in the vessel top plate and the two pumps by silicone hoses.

3.7.1 Measurement System

The pH measurement system is designed for use with HAMILTON digital sensors:

- Conventional pH sensor (potential measurement against reference) with built-in electronics

- Type: Easyferm Plus ARC
- Manufacturer: HAMILTON

INFORMATION

The matching pH sensor is included in the standard package. These pH sensors are pre-configured by the equipment manufacturer, INFORS HT. Replacement sensors must be configured before use.

For details on the technical data, maintenance and storage requirements for the pH sensors, see the separate documentation provided by the sensor manufacturer. Read and follow the instructions.

Calibration of the pH sensor is always carried out **BEFORE** autoclaving. This is done on the operating panel. For more details on this procedure refer to the main chapter “Operation”, chapter “Calibrating the pH Sensor”.

INFORMATION

If the pH sensor has already been calibrated before connection to the system, the bioreactor will use this data and calibration on the operating panel is no longer necessary.

For culture vessels with nominal widths of 90 and 145, pH sensors can be mounted directly into 12 mm/Pg13.5 ports. For culture vessels with a nominal width of 115, an electrode holder is used.

For more details on the electrode holder, see the “Accessories” section, sub-section “Electrode Holder”.

3.8 pO_2 Control

The oxygen saturation of the (culture) medium is measured by the pO_2 sensor, and can be adjusted as follows:

Increasing the pO_2

The content of the oxygen dissolved in the medium (pO_2) can be increased using the following methods:

- Increasing the stirrer speed
- Increasing the gas volume flow rate (air and/or oxygen)
- Increasing the oxygen content in the Gasmix.

These approaches can also be combined.

pO_2 reduction

In anaerobic processes, the vessel can be gassed using nitrogen. This displaces the oxygen dissolved in the medium.

3.8.1 Measurement System

The pO_2 measurement system is designed for use with HAMILTON digital sensors:

- pO_2 sensor with built-in opto-electronics
- Type: Visiferm DO ARC
- Manufacturer: HAMILTON

INFORMATION

The HAMILTON pO_2 sensors are pre-configured by the equipment manufacturer, INFORS HT. Replacement sensors must be configured before use.

For details on the technical data, maintenance and storage requirements for the pO_2 sensors, see the separate documentation provided by the sensor manufacturer. Read and follow the instructions.

Generally speaking, the following applies: Unlike measurements such as pH, which are calibrated to absolute measurement values, the oxygen measurement is always calibrated to a relative reference point. For this purpose, the calibration is set to 100 % relative oxygen saturation, usually with air at max. stirring speed and maximum gas flow rate. The actual concentration of dissolved oxygen in mmol/L may therefore vary at 100 % saturation, depending on the process.

Calibration is always carried out **AFTER** autoclaving. This is done using the operating panel. Depending on the specifications defined by the user, the pO_2 sensor will be calibrated either before the vessel is filled with medium or afterwards, in the prepared medium. For more details on this procedure refer to main chapter “Operation”, chapter “Calibrating the pO_2 Sensor”.

For culture vessels with nominal widths of 90 and 145, pO_2 sensors can be mounted directly into 12 mm/Pg13.5 ports. For culture vessels with a nominal width of 115, an electrode holder is used.

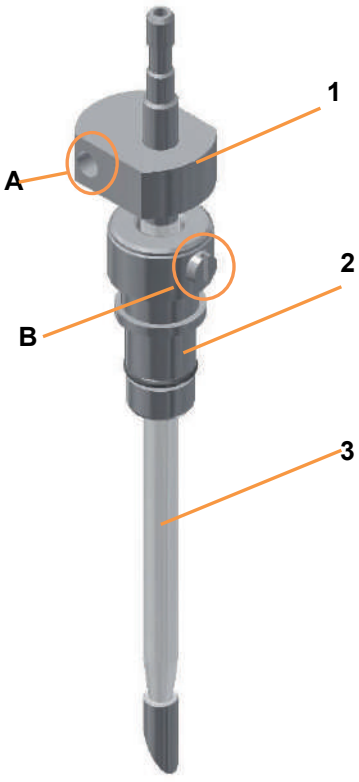
For more details on the electrode holder, see the “Accessories” section, sub-section “Electrode Holder”.

3.9 Antifoam Control

Foam hinders the exchange of gas between the medium and the gas phase in the head space. The exit gas filter can become clogged with foam, which causes a pressure build-up in the vessel. This can be prevented by adding antifoam agent.

The antifoam agent is kept in a reagent bottle that is connected to the antifoam sensor and the antifoam pump via a hose. The sensor also acts as a dosing needle. When the sensor comes in contact with foam, the antifoam pump is activated and antifoam agent is fed into the vessel via the dosing needle.

3.9.1 Antifoam Sensor



Inside diameter	2 mm
Hose connection outside diameter	4 mm

A clamping adapter with a fixed O-ring is used to mount the sensor in the 10 mm port in the vessel top plate.

1 Sensor head with port for banana connector (A)
2 Clamping adapter with slotted-head screw (B)
3 Needle with transparent insulation

The antifoam sensor is equipped with two NON-autoclavable protective caps.

4 Options

The following options are available in addition to the equipment included in the scope of supply for the basic unit.

4.1 Turbidity Measurement

The Optek ASD12-N measurement sensor can be used to determine the turbidity of the culture. Turbidity measurement can be used to draw conclusions regarding the biomass concentration in the culture.

The system comprises a sensor with a transmitter that is integrated into the bioreactor basic unit:

- Type ASD12-N with OPL05 (optical path length) for higher cell densities.
- Manufacturer: Optek
- Measures the absorption in the 0 – 4 CU range

The ASD12-N sensors supply a non-linearised turbidity measurement for the culture. This can be linearised manually using the soft sensor in eve®, for example, in order to determine correlation with factors such as the biomass concentration or optical density.

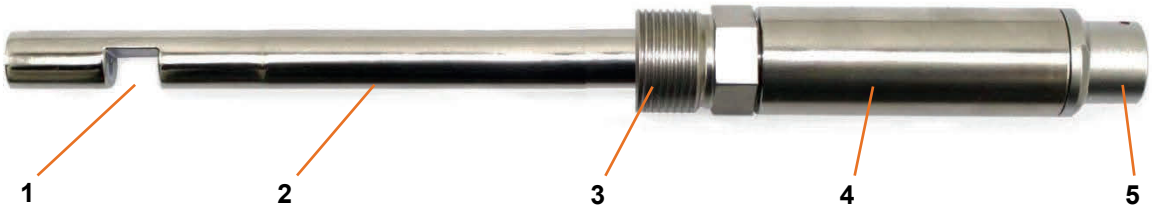
INFORMATION

The Optek ASD sensors are designed exclusively for use as turbidity sensors for liquids and gases in accordance with the technical data (“Specifications”).

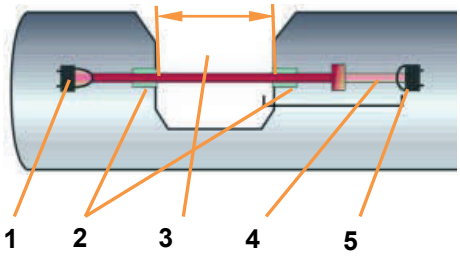
The use of these sensors is forbidden in potentially explosive areas.

4.1.1 Sensors

Optek ASD sensors are high-precision turbidity sensors. They can be used to draw conclusions regarding the growth of microbial cultures or cell cultures as a function of NIR absorption.



1 Measurement gap (optical path length, OPL)
2 Shaft, Ø 12 mm
3 PG 13.5
4 Sensor head
5 Connection (push-pull plug connection)



The sensors possess a measurement segment that is optimised in terms of flow and sterility. The seal-free design of the sapphire window means that there are no gaps or joints. This guarantees complete sterility. All components that come into contact with the media are made of electrolytically polished steel.

Main components

1 LED light source
2 Sapphire window
3 Optical path length (OPL)
4 Daylight filter
5 Silicon photodiode detector

A defined LED light beam is shone through the process medium. The weakening of the light intensity, which is caused by absorption and/or diffusion by the dissolved and undissolved particles in the carrier medium, is detected by a hermetically encapsulated silicon photodiode.

The ASD-N sensors use the light in the near-infrared range (NIR) of 840 nm to 910 nm.

4.1.2 Calibrating the Zero Point

Optek sensors are pre-calibrated in the factory. Inserts are available for reference measurement.

Due to the different light absorption of different media, zero point calibration should be performed before each cultivation process.

This can be done on the operating panel, either **before or after** autoclaving, depending on the application in question. For more details, see the main chapter “Operation” chapter “Calibrating the Turbidity Sensor”.

4.1.3 Mounting the Sensor

For culture vessels with nominal widths of 90 and 145, Optek ASD sensors can be mounted directly into 12 mm/Pg13.5 ports. For culture vessels with a nominal width of 115, an electrode holder is used. For more details on the electrode holder, see the “Accessories” section, sub-section “Electrode Holder”.

Please note the following points when mounting the components:

- Ensure that the sensor is fitted with an O-ring; fit an O-ring if necessary.
- Mount the sensor by hand – do not use any tools!
- If the mounting depth of the sensor is adjustable (mounting with electrode holder), make sure the mounting depth is set correctly prior to autoclaving, as later adjustment represents a contamination risk.
- Mount the sensor in such a way that it cannot come in contact with other components or the glass vessel.
- Mount the sensor in such a way that it has good access to the flow and there is no risk of bubbles collecting in the measurement gap.

4.1.4 Cleaning and Storing the Sensor

A build-up of deposits on the sapphire window will cause shifts in the zero point and restrict the dynamic range of the sensor. As such, the sapphire window on the sensor must be checked for deposits before each use and cleaned if necessary.

If necessary, store the Optek sensor in a clean, dry place at a temperature of between -20°C and 70°C.

4.1.5 Interferences Turbidity Measurement

Interference		
Displayed measured value is not plausible / unusual		
Possible Cause	**Remedy**	**By**
Sensor cable is twisted or kinked or not properly connected.	Check and ensure that the sensor cable is not kinked or twisted. Connect the sensor cable properly as necessary.	Operator
Sensor is not calibrated	Calbrate the zero point	Operator
Window fouling on the sapphire windows .	Carefully clean the sensor	Operator
Faulty sensor cable	Replace sensor cable	Qualified electrician
Faulty sensor	Replace the whole sensor	Operator

INFORMATION

The measuring system automatically switches off, if the temperature of the sensor in the medium exceeds 50 °C during operation (thermal shutdown). Once the medium has cooled down, measurement automatically continues.

4.1.6 Specifications

Description	Value	
Sensor type and manufacturer	ASD12-N	
Path length	OPL05: for higher cell densities	
Material sensor	316L, electropolished and sapphire glass	
Measuring principle	1-channel absorption of light	
Measurement wavelength	840 nm – 910 nm	
Detector	Silicone photodiode (hermetically sealed)	
Measuring range	0 – 4 CU (concentration units)	
Protection	IP 68	
Light source	Hybride LED	
Temperature	Shutdown at	50 °C[1]

1) *The measuring system automatically switches off, if the temperature of the sensor in the medium exceeds 50 °C during operation (thermal shutdown). Once the medium has cooled down, measurement automatically continues.*

4.2 Exit Gas Analysis

In order to allow the user to draw conclusions regarding the status of the culture while the bioprocess is still underway, the CO_2 and O_2 measurements are often taken and analysed in the exit gas flow of the bioreactor.

4.2.1 Gas Sensors

Combined CO_2 and O_2 gas sensors of the type BlueInOneFerm or Blue Vary from the manufacturer BlueSens are available for exit gas analysis.

3 m of pressure hose, D = 8 x 14.5 and a clamp are included for connecting the gas sensor to the culture vessel (exit gas filter).

Measurement ranges gas sensors

Vol. % O2	Vol. % CO2
1.0 – 50	0 – 10
	or
	0 – 25

INFORMATION

Both sensor types are only suitable for use in aerobic bopro-cesses!

For details on the safety, technical data, usage and maintenance requirements for the gas sensors, see the separate documentation provided by the sensor manufacturer. Read this documentation before using the gas sensor and follow the instructions contained therein.

4.2.2 Connecting the Gas Sensor

In order to view measurements on the operating panel, you must connect the gas sensor to the sensor cable and channel the exit gas from the bioreactor through the gas sensor using a hose. The cable is usually connected once during commissioning and can remain untouched thereafter. The connection to the exit gas line must be re-established before each cultivation process.

The ideal connection conditions are detailed in the separate docu-mentation provided by the manufacturer.

Connecting the sensor cable

The fixed sensor cable is pre-installed in the factory (rear of equipment). The cable has an 8-pin round plug connector. In order to connect the sensor, the plug connector is plugged into the socket marked Port **A** on the gas sensor.

Due to the length of the sensor cable, the gas sensor can be positioned in a large number of possible locations.

Establishing the hose connection

The hose connection between the culture vessel (exit gas filter) and the gas sensor must be designed in line with the direction in which the gas flows through the gas sensor.

Proceed as follows:

Procedure

1. Cut as short a piece as possible off the supplied pressure hose.
2. Push one end of the hose onto the hose nozzle (observe direction of flow) on the gas sensor's flow adapter and fasten in place with the clamp.
3. Push the open end of the hose onto the exit gas filter on the exit gas cooler.

INFORMATION

Do NOT use a clamp here, as the hose must be easy to disconnect at this point, e.g. for autoclaving the culture vessel.

4.2.3 Calibrating the Gas Sensor

1-point calibration must be carried out once per month and during initial commissioning in order to guarantee exact measurement results.

This is done directly on the gas sensor itself. The procedure is described in the separate documentation provided by BlueSens.

4.2.1 Replacing the BlueVary Gas Sensor Cartridge

The max. operating time of a BlueVary gas sensor cartridge amounts to 9000 operating hours. Once this limit is reached, measurement is no longer possible. I.e. there is no measured value output anymore and the display turns red. The gas sensor cartridge must be replaced by the sensor manufacturer.

For detailed information refer to the separate documentation from the sensor manufacturer.

5 Accessories

The table below lists all the accessories included in the standard package, divided according to vessel size (TV = total volume) and nominal vessel width (inside diameter).

Accessories	1.5 L TV/NW 90	3.0 L TV/NW 115	6.0 L TV/NW 145
Impeller, Rushton	2	2	2
Baffles	1	1	1
Sparger, ring-shaped	1	1	1
Pocket for temperature sensor in 10 mm port	1	1	1
Dip tube, straight, Ø 6 mm for 12 mm/Pg13.5 port	1	1	1
Addition port adaptor, for 7.5 mm port	4	4	4
Clamping adapter for 10 mm port	3	3	3
Antifoam sensor for 10 mm port	1	1	1
Blanking plug for 12 mm/Pg13.5 port	4	6	7
Blanking plug for 10 mm port	2	2	2
Exit gas cooler for 12 mm/Pg13.5 port	1	1	1
Reagent bottle, 250 mL	4	4	4
Pump heads with hoses Inside diameter: 1.0 mm/wall thickness: 1.1 mm	4	4	4
pO_2 sensor, Visiferm DO	1	1	1
pH sensor, Easyferm	1	1	1
Starter kit	1	1	1
Electrode holder for 12 mm/Pg13.5 port	2	2	2
Cone plug for drive hub	1	1	1

5.1 Cone Plug for Drive Hub

The cone plug (EPDM) provided in the starter set protects the drive hub from penetration of condensate water during sterilisation in the autoclave.

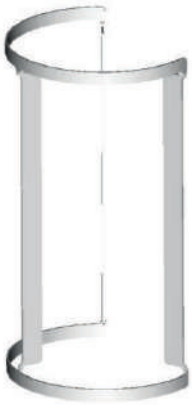
It must be plugged into the opening of the drive hub for autoclaving of the culture vessel!

5.2 Sparger

The gas is fed directly into the medium via a ring sparger (Ø 6 mm). The sparger is mounted in a 10 mm port in the vessel top plate using a clamping adaptor, and connected to the gassing system on the basic unit via a silicone hose with a sterile filter.

Inside diameter	4.0 mm
Hose connection outside diameter	6.0 mm

5.3 Baffles



The baffles are used to mix the culture. They are simply inserted into the glass vessel.

5.4 Blanking Plugs

Blanking plugs are used to seal open ports. There are different blanking plugs for the different types of port.

Blanking plug, Ø 10 mm

Fitted with fixed O-ring.
A fastening screw is used to fasten it in the 10 mm vessel top plate port (see the “Clamping Adaptors and Fastening Screws” section).

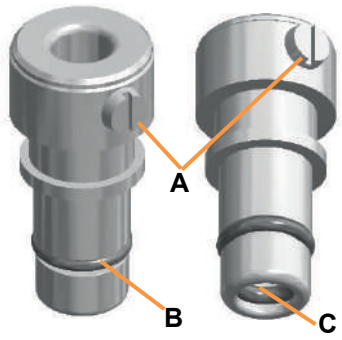
Blanking plug, Ø 12 mm

Must be fitted with an O-ring before being mounted in the 12 mm/Pg13.5 port.
Mounted using a thread.

5.5 Clamping Adapters and Fastening Screws

Clamping adapters are used when mounting the sparger, the various dip tubes and the antifoam/level sensors. The clamping adapter fixes the component in place and can be used to adjust its mounting depth.

The clamping adapter must match the outside diameter of the part being installed and the size of the port in the vessel top plate.



Clamping adapter Ø 6/10 mm

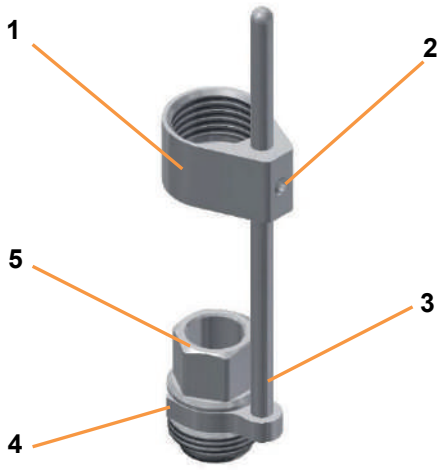
Fitted with two fixed O-rings (B & C).

When the slotted-head screw is undone, the component with a diameter of 6 mm can be inserted in or removed from the clamping adapter. When the slotted-head screw is tightened, the component is clamped in the clamping adapter.

Fastening screw M5

The fastening screws are used to hold components in place in the Ø 10 mm ports in the vessel top plate.

5.6 Electrode Holder

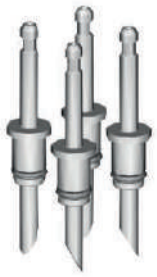


Electrode holders are used for mounting sensors (pH, pO_2, etc.) in 12 mm/Pg 13.5 ports. They can also be used to adjust the mounting depth of the sensor.

The electrode holder comprises a sheath with a grub screw, a guide bar with a fork, and a hollow screw. The wrench for the grub screw is also included in the scope of supply.

1 Sheath
2 Grub screw
3 Guide bar
4 Fork
5 Hollow screw

5.7 Addition Port Adapters and Feed Needles



Addition port adapters and feed needles are used to feed liquid into the culture vessel. They each come with a tubing connection, are fitted with a fixed O-ring and are mounted into the four 7.5 mm ports in the vessel top plate. A single fastening screw is used to fasten all four addition port adapter(s) and/or feed needle(s) in place.

Addition port adapter Ø 7.5 mm

Inside diameter	2 mm
Hose connection outside diameter	4 mm
Installation depth	17 mm

Addition port adapters protrude as far as the head space of the vessel, and have very sharp ends with slanted points.
Each culture vessel comes with four addition port adapters as standard.

Feed needle Ø 7.5 mm

Inside diameter	2 mm
Hose connection outside diameter	4 mm

Feed needles protrude to below the minimum fill level (= min. working volume) of the culture vessel.
This method of adding liquid allows more precise and regular dosing even when handling small volumes as, unlike the port adapter, the feed needle does not drip.

INFORMATION

The illustration on the left shows only the upper section of the feed needle.

5.8 Septum Collar

The septum collar with inside thread is used to inoculate the culture in combination with the syringe, injection needle and septum (inoculation membranes); see the “Inoculation Accessories” section. The septum collar is used to hold the septum in place in the 12 mm/Pg13.5 port.

5.9 Dip Tubes

Dip tubes are open at both ends and are mounted in a vessel top plate port with a clamping adapter.

Dip tubes are used for a variety of purposes:

- For filling the culture vessel after autoclaving. Using a dip tube prevents foaming.
- For adding inoculum.
- For sampling. The aseptic Super Safe Sampler system can be used for sampling.
- For harvesting
- For siphoning off medium during continuous cultivation
- For draining the culture vessel

Depending on the purpose, silicone hoses are connected to the dip tube via other vessels, sampling systems or, if necessary, hose networks.

Multiple dip tubes can be used at any one time, providing that enough vessel top plate ports are available.

Dip tube, straight, Ø 6 mm

Inside diameter	3.0 mm
Hose connection outside diameter	4.0

The dip tube does not reach as far as the bottom of the vessel.

INFORMATION

The illustration on the left shows only the upper section of the dip tube.

5.10 Pocket for Temperature Sensor (Pt100)

The pocket is a pipe with a sealed bottom end, and is used to insert the temperature sensor.

Pocket Ø 10 mm

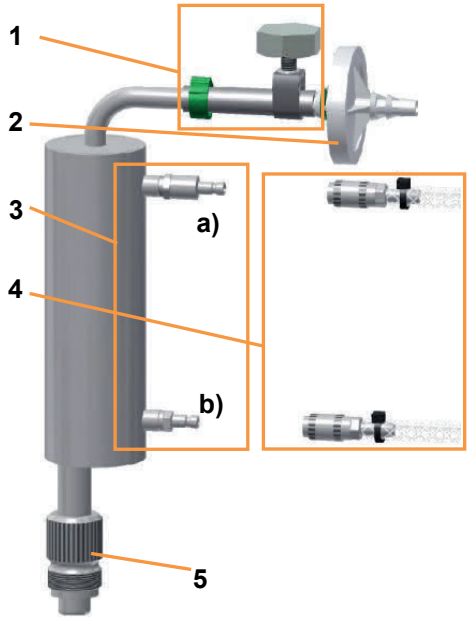
Fitted with fixed O-ring.
A fastening screw is used to fasten it in the 10 mm vessel top plate port.

The diagram on the left does not show the fill length of the pocket.

5.11 Exit Gas Cooler

The basic unit supplies cooling liquid to the exit gas cooler. The cooling liquid flow rate can be adjusted using the control valve on the basic unit. The two hoses for water supply (bottom) and return (top) are connected to the basic unit at the factory and simply connected to the exit gas cooler via the two rapid couplings.

A piece of pressure hose is fitted onto the exit gas cooling pipe and equipped with an exit gas filter. The hose connections and the exit gas filter are secured with hose clamps. The hose clamp securing the exit gas filter is equipped with a screw that can be used to loosen or tight it manually.



1 Pressure hose und hose clamp
2 Exit gas filter
3 Hose connections:
   a) Water outlet
   b) Water inlet
4 Hoses with rapid couplings for water supply and return (connected before delivery!)
5 Screw thread

The exit gas cooler is fitted with an O-ring before installation.
A screw thread is used to mount it in the 12 mm/Pg13.5 vessel top plate port.

INFORMATION

The exit gas filter must be replaced with a new filter after each cultivation process.

The exit gas cooler only works when the temperature control system is switched on.

5.12 Reagent Bottles

Two sizes of borosilicate reagent bottle are available for adding reagent and feed solution:

- 250 mL
- 500 mL

Reagent bottles are fitted before delivery. There are two hose connections on the lid. One is fitted with a short piece of silicone hose with a filter for pressure equalisation.
The second connection is fitted with a piece of silicone hose at the other end, inside the bottle.
A 2 m piece of hose is included in the scope of supply for connecting the reagent bottle to the addition port adapter in the culture vessel and to a pump head.

1 Filter
2 Silicone hose Ø = 2 x 6 mm
3 Cable tie

INFORMATION

250 mL reagent bottles are supplied as standard in the unit package. These fit in the reagent bottle holder that is built into the vessel holder.

Before delivery with the equipment, the reagent bottles are fitted with filters and hoses of the correct length, and connected to the pump heads.

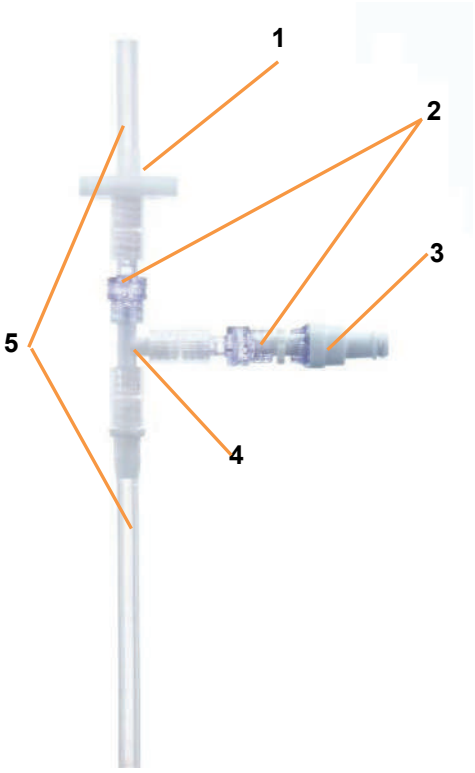
5.13 Sampling System Super Safe Sampler

Basically different systems and also individual components are available for sampling. This operating manual describes the operation and handling of the aseptic sampling system Super Safe Sampler combined with a dip tube.

The use of the Super Safe Sampler prevents the culture vessel from contamination when sampling.

Content of the set

The set consists of a completely pre-assembled group of valves with hoses and two syringes. It is connected via silicone hose with a dip tube.

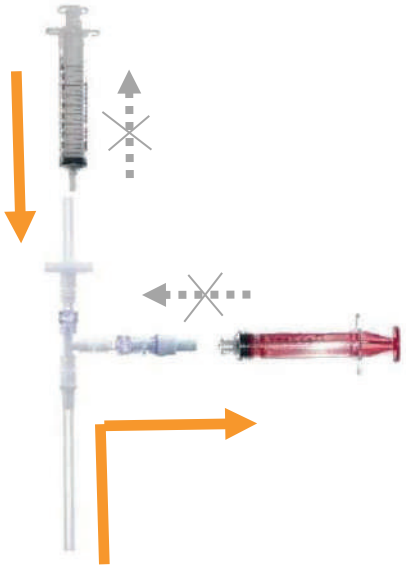


Valve assembly

1 Sterile filter
2 Check valve
3 Luer activated sample valve
4 T-piece
5 Hose

The valve assembly consists of a T-piece, two check valves, a Luer-activated automatic sample valve, a sterile filter, a length of hose as an adapter for the syringe and another hose for connection to the sample dip tube in the culture vessel.

Accessories



Principle of function

The sample valve on the side arm of the T-piece opens by putting the Luer connector of the syringe into the valve and closes by removing the syringe. No further handling is necessary.
Unintentional re-introduction of the sample material once it has been withdrawn is prevented by a check valve. Thus, contamination of the bulk culture is impossible.

Following sampling, a second syringe can be fitted and air pushed in via the sterile filter, in order to displace culture solution from the sample hose and the dip tube of the vessel. With a conventional sampling system, the next sample cannot be taken immediately, as rinsing of the sampling hose and the immersion tube is necessary. By previously removing most of the culture in the sampling line, this sampling system can save culture volume, which is particularly important with small vessels and/or frequent sampling.

The dead volume of the culture remaining in the group of valves after flushing with sterile air amounts to a few µl and is negligibly small. If the withdrawal of a very small sample volume is required, with minimum possibility of falsification, a small quantity of culture solution (e.g. 1 ml) can be introduced and rejected before the actual sample is taken.

Designated use

The Super Safe Sampler is designed for aseptic sampling of completely liquid samples.

Solid parts in the sample may lead to clogging of the valves. Therefore, employing the Super Safe Sampler for solid media is not recommended.

The Super Safe Sampler is autoclavable (not the syringes!) and for this reason reusable.

Practical tips for the use of the Super Safe Sampler

Sterility of the culture vessel is ensured at all times without the possible measures mentioned below.
The use of a sterile syringe and sterile caps is only necessary if the

sample has to be processed under sterile conditions. For sampling, the same non-sterile syringe can be used repeatedly, without fear of contamination of the culture vessel.

Aseptic Sampling

For each sample, use a new, sterile syringe with Luer Lock fitting, in order to ensure the sterility of the sample.

Sterile syringes are consumables and therefore not included in the set.

INFORMATION

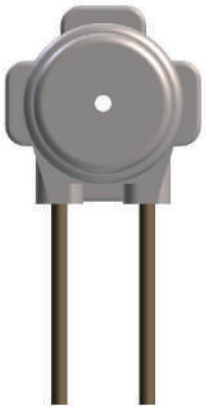
The use of another syringe is also possible. But a syringe with Luer lock prevents unwanted movement of the syringe.

- Before fitting the syringe, disinfect the sample valve. Fort this, spray a commercially available disinfectant onto the valve.
- After spraying and after each sampling, close the the sample valve with a sterile Luer-Lock cap (Dead End Cap) to keep the valve and sample sterile.

The caps are not included in the kit. Very convenient to use are so-called combi-caps that fit on male and female connectors alike.

Caps that are vented and made of steam sterilisable material can also be fitted during autoclaving.

5.14 Pump Heads

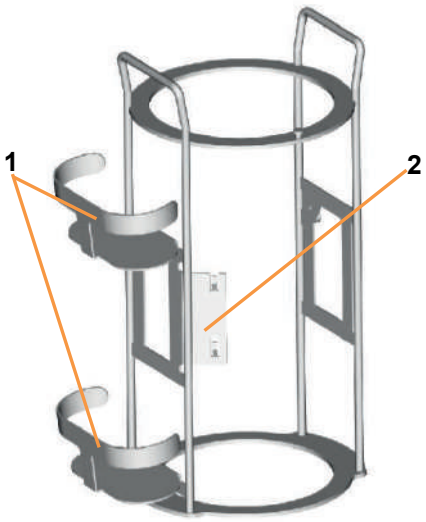


The autoclavable pump heads are fitted with PharMed pump hoses prior to delivery. Three different hose diameters are available for different delivery rates:

- 1.0 mm (standard)
- 0.5 mm
- 2.5 mm

For more detailed information about pumps and hoses refer to main chapter “Technical Data”, chapter “Specification”, “Pumps

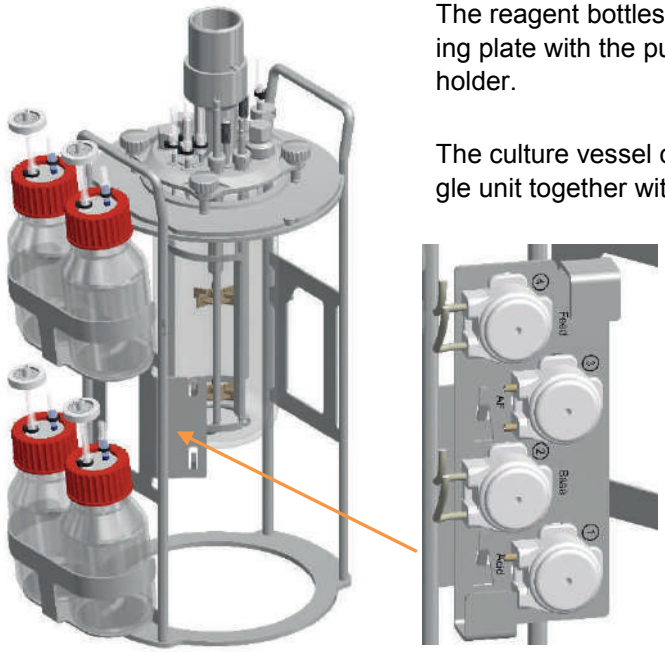
5.15 Vessel Holder with Built-in Holder for Reagent Bottles and Pumps



The vessel holder frame has a holding device for four reagent bottles and a holder for the four pump heads.

1 Reagent bottle holder

2 Pump holder



The reagent bottles are placed in the two holders, and the mounting plate with the pump heads is simply pushed onto the pump holder.

The culture vessel can this be transported and autoclaved as a single unit together with the reagent bottles and pump heads.

5.16 Sterile Filters

Sterile filters are used to protect against contamination in both the gassing line and the exit gas line. In addition to this, all reagent bottles used for pressure equalisation must be fitted with an open, short section of hose with a sterile filter.

Accessories

All the sterile filters in the scope of supply are autoclavable, disposable filters with PTFE diaphragms.

INFORMATION

Sterile filters must be clean and dry at all times, and should thus ideally be replaced after each use.

Ø 37 mm, marked red

Application	Inlet air (sparger) 1.5 lit. culture vessels
Retention rate	0.2 µm

Ø 50 mm, Type ACro50 TF, no label

Usage	Inlet air (sparger) 3.0 and 6.0 L culture vessels
Retention rate	0.2 µm

Ø 37 mm, marked green

Application	Exit gas
Retention rate	0.3 µm dry 1.0 µm wet

Ø 25 mm, not marked

Application	Super Safe Sampler
Retention rate	0.2 µm
Diaphragm	PTFE

Accessories

Ø 25 mm, not marked

Application	Reagent bottles (pressure equalisation)
Retention rate	0.45 µm
Diaphragm	PTFE

5.17 Hoses and Accessories

The following hoses and accessories, such as clamps and brackets, are available:

Hose type	Ø	Application
Pressure hose, fibreglass-woven	6 x 11.9 mm	Water and gas connections (on-site)
Pressure hose, fibreglass-woven	6 x 10 mm	Exit gas filter attachment (on exit gas cooler)
Pressure hose, transparent	5 x 10 mm	Inlet air filter attachment on sparger for 3.0 and 6.0 L TV culture vessels
Silicone hose	5 x 8 mm	Gassing (sparger)
Pressure hose, transparent	4 x 8 mm	Water supply and return, exit gas cooler
Silicone hose, transparent	2 x 6 mm	Reagent bottles

Attachments	Application
Clamp, 12 mm, INOX	Water and gas connections (on-site) Exit gas filter attachment (on exit gas cooler)
Hoffmann pinchcock, 12 mm, nickel-plated brass	Unclamping hose lines, e.g. unused addition port adaptors/feed needles, sparger hose line, etc.
Cable tie, 2.4 x 86, polyamide	Hoses for reagent bottles and pumps, inlet air filter, sparger, water supply and return for exit gas cooler, sampling system dip tube

Hose connectors	Application
Double hose nipple, 3/32" x 1/16", PVDF	Pump heads with hoses to reagent bottles

5.18 O-Rings and Gaskets

Designation	Ø [mm]	Application
O-ring, EPDM	3.53 x 94.84	Top plate gasket, culture vessel, NW90
O-ring, EPDM	3.53 x 120.24	Top plate gasket, culture vessel, NW115
O-ring, EPDM	3.53 x 148.8	Top plate gasket, culture vessel, NW145
O-ring, EPDM	2.62 x 10.77	Gasket, port size 12 mm/Pg13.5
O-ring, EPDM	1.5 x 7.5	Gasket, port size 10 mm
O-ring, EPDM	1.5 x 5.0	Gasket, port size 7 mm
O-ring, EPDM	1.78 x 5.28	Inner gasket for clamping adaptor for 10 mm ports
PTFE ring	120 x 105	Damping ring between glass vessel and vessel holder, NW90
PTFE ring	145 x 130	Damping ring between glass vessel and vessel holder, NW115
PTFE ring	175 x 160	Damping ring between glass vessel and vessel holder, NW145
Flat gasket, silicone	32 x 42 x 2	Gasket for reagent bottle lid (all sizes)

5.19 Inoculation Accessories and Tools

The following inoculation accessories and tools are used:

Accessories for inoculation
Septum (inoculation diaphragm), Ø = 16 mm MVQ silicone, transparent, for 12 mm/Pg13.5 ports
Sterile disposable syringe, Luer, 10 mL, inside Ø 14.35 mm
Sterile hollow needle, 20G, L = 40 mm/Ø = 0.9 mm

Tools for screws, grub screws and blanking plugs
Allen key, WAF 2, DIN911 For grub screws on impellers
Allen key, WAF 1.27 For grub screws

Hexagon socket spanner, WAF 17 For blanking plugs in 12 mm/Pg13.5 ports	
Torx wrench, TX25 For screws on the thermal block adapter	

5.20 Starter Kit

Each equipment package comes with a starter kit with a variety of hoses, attachments, inoculation accessories and tools. A detailed contents list is included in each starter kit.

5.21 Service Sets

Service sets with O-rings, gaskets, sterile filters, hoses, etc. to fit each vessel size are available separately. A detailed contents list is included in the service set.

5.22 Auxiliary Supplies

The term “auxiliary supplies” covers all the substances and materials required for operation and/or maintenance that cannot be considered part of the equipment or the system.

pH Buffers

pH buffers are used to calibrate the pH sensors. 250 mL bags are available for the following buffers:

- pH 4.04
- pH 7.01

6 Transport and Storage

The following specifications are based on transport and storage of an unpacked equipment at the provider's site.

6.1 Transport

WARNING

Improper transport, the use of incorrect auxiliary equipment and careless handling of the equipment may lead to injuries and severe property damage.

The following points must be observed when transporting the equipment internally (relocation):

- Always work in pairs and use suitable auxiliary equipment when transporting the equipment.
- The entire equipment (basic unit and culture vessel) contains delicate glass parts.
- Especially when using auxiliary tools, it is important to observe that the equipment's centre of gravity is not in the middle.

WARNING

The entire equipment (basic unit and culture vessel) is too heavy to be carried by one person alone.

Even the basic unit on its own exceeds the weight that should be carried by one person alone.

6.2 Storage

- Before each time they are put into storage, decontaminate, thoroughly clean and dry the culture vessel and all accessories[1].
- Store the equipment and its components clean, dry and protected against dust, dirt and liquids.
- Store the equipment and its components in a cool place with low air humidity but protected against frost.
  - Storage temperature: 5°C – 55°C
  - Relative air humidity, non-condensing: 10% - 95%.
- Protect the equipment from aggressive media, direct sunlight and mechanical vibrations.

1) *Maintain and store sensors produced by other manufacturers in accordance with the separate documentation.*

7 Installation and Initial Operation

To set up and connect the equipment, note the following:

⚠ WARNING

Faulty installation may lead to dangerous situations or severe loss of property.

Follow the installation and commissioning instructions in this operating manual precisely.

7.1 General Location Requirements for Installation

The following requirements must be met for the installation of the equipment:

- The figures and ranges specified in the chapters “Technical Data, Connection Values” and “Technical Data, Operating Conditions” must be observed.
- The equipment must only be installed inside a laboratory or a laboratory-like environment.
- The installation site must be level, sufficiently stable and able to bear loads.
- There must not be any sources of electrical interference near the equipment.

7.2 Minimum Distances

To operate and maintain the unit it must be installed with a minimum spacing of 150 mm from walls, ceilings or other equipment.

7.3 Connecting the Equipment to On-Site Supply Lines

The following chapters describe which connection requirements must be fulfilled on site and how the equipment is connected to on-site supply lines.

7.3.1 Power Supply

Connection conditions

The in-house power supply to the equipment must meet the following requirements:

- Single-phase, constant power supply
- 120/230 VAC 50/60 Hz

Connection

To connect the basic unit to the in-house power supply, proceed as follows:

Procedure

1. Insert the power cable supplied into the connector socket on the equipment.
2. Insert the cable into the in-house power supply.

7.3.2 Water Supply and Return

Connection conditions

The in-house water supply to the unit, as well as the drainage of the water, must meet the following requirements:

- "Very soft" or "soft" water quality ($CaCO_3$ concentration 0 mmol L^{-1} to 1.5 mmol L^{-1})

ATTENTION

Not observing the water quality requirements may lead to damage or failure of the equipment.

- Constant water supply at a pressure of 2 ± 1 bar
- Manometer to check the primary pressure available
- The drain is heat-resistant and without back pressure

Connection

To connect the basic unit to the in-house water supply and drainage, proceed as follows:

Procedure

1. Cut the required quantity of the supplied pressure hose (Ø = 6 x 11.9 mm).

2. Position the pressure hoses on the appropriately marked hose nozzles on the basic unit.
3. Connect the hoses to the in-house water supply and drainage.
4. Secure the hoses with hose clamps to prevent slipping.
5. Check to ensure that the hoses neither have kinks nor are able to kink and that connections and hoses do not have any leaks.

7.3.3 Gas Supply

Connection conditions

The in-house gas supply to the equipment must meet the following requirements:

- Constant gas supply at a pressure of 2 ± 0.5 bar
- Gas(es) is/are dry, clean and free of oil and dust
- Recommended compressed-air quality as per DIN ISO 8573-1: Class 1,2,3,4

ATTENTION

The use of impure gases can lead to blockage of the sterile filter and damage the mass flow controller.

Only use dry, clean and oil-free gases.

Connection

To connect the basic unit to the in-house gas supply, proceed as follows:

Procedure

1. Cut the required quantity of the supplied pressure hose (Ø = 6 x 11.9 mm).

   Only use hoses supplied by the manufacturer.
2. Position the pressure hoses on the appropriately marked hose nozzles on the basic unit.
3. Connect the hoses to the in-house gas supply.
4. Secure hoses with hose clamps to prevent slipping.
5. Check to ensure that hoses neither have kinks nor are able to kink and that connections and hoses do not have any leaks.

WARNING

The use of inappropriate or damaged hoses and/or inappropriate fixing may lead to leakage of gases. Depending on the gas in question, there may be a danger of gas explosion and/or danger of suffocation as well as a hazard for the health of the operator.

Always close the gas supply before a hose is removed and when the equipment is not in use.

7.3.4 Exit Gas

On site, it must be ensured that:

- the exit gas is dissipated securely by means of a suitable, gas-tight hose.
- the working environment and/or the laboratory/laboratory-like facility is equipped with a sufficient ventilation system, depending on the application.

7.4 Connecting the Motor Cable

The motor is controlled directly via the basic unit and is connected to it via the motor cable.

For routine operation, it is not necessary to plug in and unplug the motor cable. The connected motor is only coupled before cultivation. For details, see the main chapter “Before Cultivation” and the “Connecting the Motor” chapter.

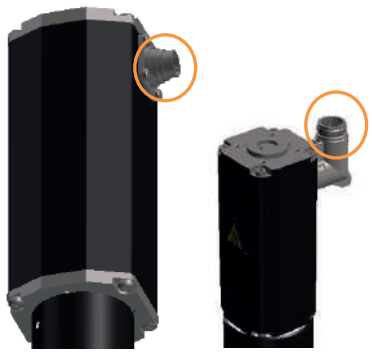
ATTENTION

If the motor cable is connected to or disconnected from the motor while the equipment is switched on, there is a risk of a short circuit that could damage the control electronics.

To connect the motor cable, proceed as follows:

Procedure

1. Insert the (angled) plug of the motor cable into the socket on the rear of the basic unit.



2. Insert the other plug into the socket on the motor.

   Depending on the size of the culture vessel, the large (left-hand) or small (right-hand) motor is supplied.

7.5 Test Run

In order to become familiar with the basic functions of the equipment before the first cultivation, a short test run can be executed. The test run comprises:

- Temperature control (cooling / heating)
- Stirring
- Gassing

Compressed air of the stated quality (see chapter “Gas Supply”) is used for gassing.

To avoid calcium deposits, demineralised water is recommended for filling the vessel.

The following description of the test run does not detail handling of individual components, e.g. exit gas cooler, stirrer, sparger etc. Detailed descriptions of their handling are given in the corresponding chapters of the main chapter “Before Cultivation”.

For details on operation, see the main chapter “Operation”.

7.5.1 Preparation Test Run

Before starting the test run, check and ensure the following:

- the equipment is correctly connected to the water, power and gas supply and is operational
- the motor cable is connected to the basic unit and the motor.

The following work is to be executed before the test run:

Procedure

1. Remove the vessel top plate and put it aside carefully.

ATTENTION

If the vessel top plate presses against long components such as the stirrer shaft etc., they could bend because of the weight of the top plate.

Always position the vessel top plate so that it does not lie on top of components.

2. Fill the culture vessel with water – preferably demineralised – to the working level.
3. Ensure that the stirrer and sparger are mounted; if necessary, mount them.
4. Fit the top plate and secure it.
5. Screw the exit gas cooler into the port on the vessel top plate port.

   The exit gas cooler is equipped with a new exit gas filter in the factory.
6. Connect the exit gas cooler to pre-fitted hoses on the basic unit; to this end, follow the symbols on the basic unit:

   water inlet on bottom of exit gas cooler / water outlet at top of exit gas cooler.
7. Close all remaining open ports with blanking plugs.
8. Hang the culture vessel on the basic unit.
9. Connect the sparger to the gassing system (compressed air) on the equipment; to this end, attach the gassing hose to the nozzle on the inlet air filter.

   The sparger is equipped with hose and the inlet air filter in the factory.

   The gassing hose is attached to the basic unit in the factory.
10. Insert the temperature sensor as far as it will go into the pocket in the top plate.
11. Couple the motor.
12. Switch on the equipment at the main switch and wait until it has started up.

7.5.2 Cooling System

To activate the cooling system, proceed as follows:

Procedure

1. On the operating panel, set a low setpoint for the *Temperature* parameter, e.g. 10 °C, in order to activate the water supply to the temperature control system.

2. Start the Batch (process) using **Start Batch** and switch on the *Temperature* parameter.
3. All parameters except for *Temperature* remain switched off; switch them off if necessary.

   You should now hear water flowing into the temperature control system.

   The water supply to the exit gas cooler should be activated, too now.
4. Use your hands to check whether the exit gas cooler and thermal block and/or adapter are beginning to cool down.

   As soon as the temperature control circuit is full, water will flow out of the water outlet (*H2O OUT*) of the basic unit.

For the rest of the procedure, allow the Batch to run with the temperature switched on.

7.5.3 Stirring

Batch (process) is running with temperature switched on

To test the stirrer, proceed as follows:

Procedure

1. On the operating panel for the *Stirrer* parameter, set a low setpoint, e.g. 200 min^{-1}.
2. Switch on the *Stirrer* parameter.

For the rest of the procedure, allow the Batch to run with the temperature switched on and the stirrer running.

7.5.4 Heating and Adjusting Temperature

Batch (process) is running with temperature switched on and stirrer running

To test the heating and adjust the temperature, proceed as follows:

Procedure

1. On the operating panel, set a high setpoint for the *Temperature* parameter, e.g. 45 °C.

   The water supply for cooling is stopped; the system heats up.

⚠ CAUTION

Risk of minor burns if the heated thermal block and thermal block adapter are touched!

2. Wait until the temperature has adjusted to the setpoint.

For the rest of the procedure, allow the Batch to run with the temperature switched on and the stirrer running.

7.5.5 Gassing

Batch (process) is running with temperature switched on and stirrer running

To test the gassing, proceed as follows:

Procedure

1. On the operating panel for the *AirFlow* parameter, set a low setpoint, e.g. 1.0 L min^{-1}.
2. Switch on the *AirFlow* parameter.

INFORMATION

The maximum airflow depends on the size of the culture vessel and the correct setting in the *Vessel Type* menu.

3. Ensure that all other gas parameters (*Gas2Flow, TotalFlow, GasMix*) are switched off.

   If the gassing is working, air bubbles now form in the water in the culture vessel.

7.5.6 End of Test

After all parameter setpoints have been reached, the test can end here. The inoculation that now takes place during normal operation is not relevant to the test run.

Proceed as follows:

Procedure

1. On the operating panel, press **Inoculate** and then **Stop Batch** to stop the Batch (process).
2. Switch the equipment off at the main switch.
3. Shut off the supply lines.
4. Let the motor cool down.

CAUTION

Risk of minor burns if the motor, which heats up during operation, is touched!

When the motor has cooled down:

5. Uncouple the motor from the vessel and place it on a clean and dry work surface.
6. Empty the culture vessel.

8 Before Cultivation

The following chapters describe all the preparatory work before starting the cultivation process. This essentially comprises:

- Preparing and autoclaving the culture vessel:
  - Checking the gaskets (O-rings) on component parts and culture vessel
  - Mounting component parts
  - Filling or moistening the culture vessel
  - Preparing sensors and other accessories
  - Autoclaving
- Connecting the culture vessel and preparing for cultivation:
  - Hanging the culture vessel into place on the basic unit and connecting cables and hoses between the culture vessel and the equipment
  - Filling the vessel if necessary
  - Preparing sensors and other accessories

8.1 Preparing and Autoclaving the Culture Vessel

All accessories required for later cultivation must be prepared and mounted accordingly and autoclaved together with the culture vessel.

Certain accessories are already mounted when the equpment is delivered.

8.1.1 Checking Gaskets (O-Rings)

O-rings are used to seal all openings on the vessel and top plate. The top plate and all internal fittings or connections are thus equipped with O-rings. Before every use, the O-rings must be checked that they are present, undamaged and correctly seated. Damaged O-rings must be replaced.

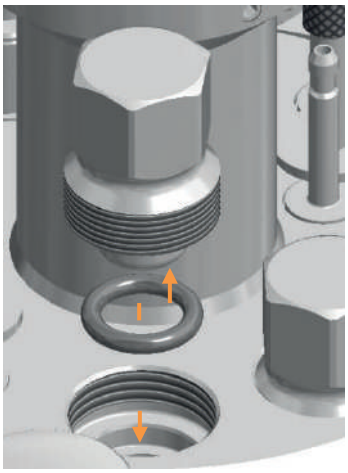
INFORMATION

Wet the O-rings with 70% alcohol or a little water to facilitate removing and replacing O-rings or component parts with O-rings. Do not use silicone grease; this can affect sterilisation results.

Carry out this check as follows:

Procedure

1. Check the O-ring for sealing the top plate for damage and to ensure that it is positioned correctly in the groove on the vessel flange.



2. Ensure that every component part is equipped with an intact O-ring:

   Check that the O-rings are correctly positioned and are undamaged. If necessary, reposition or replace. If component parts are fitted into other component parts (clamping adaptor), there must also be an O-ring between them.

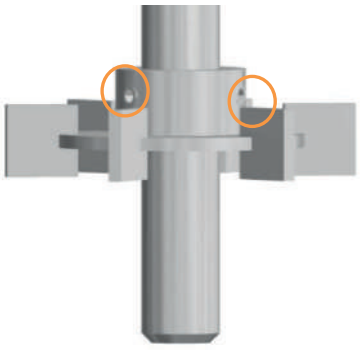
INFORMATION

Septum collars are sealed with a septum. No O-ring is used!

8.1.2 Mounting the Impellers

To mount the impellers to the stirrer shaft, proceed as follows:

Procedure



1. Slide the impeller onto the stirrer shaft.
2. Set the desired height.
3. Tighten the grub screws on the impeller with the Allen key.

INFORMATION

To avoid unnecessary foam formation, do not fit the impeller at the same height as the surface of the medium.

8.1.3 Mounting Dip Tubes and Spargers

Straight spargers and dip tubes can be mounted to the outside of the vessel top plate. Curved spargers and dip tubes can only be mounted to the inside of the vessel top plate.

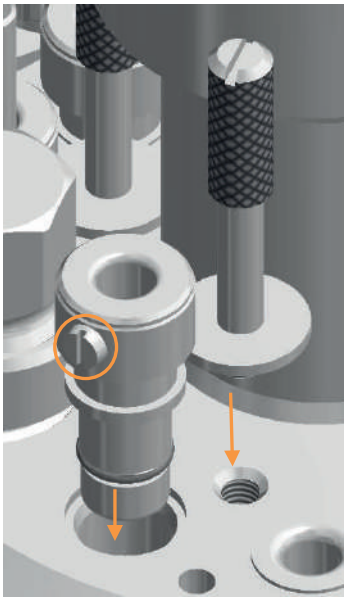
Since this unit uses curved spargers and straight dip tubes, mounting to the inside of the vessel top plate is described here. This means that the vessel top plate is still removed.

During mounting, ensure that the sparger or the dip tube does not come into contact with other component parts (stirrer). The sparger is positioned below the stirrer shaft.

Proceed as follows:

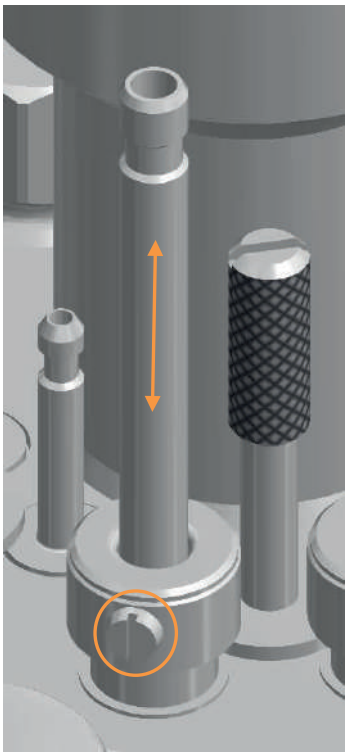
Procedure

1. Ensure that the clamping adapter is equipped with an interior or exterior O-ring; if necessary, attach O-ring(s).



2. Insert the clamping adapter into the intended port and fix it with a fastening screw.
3. Loosen the slotted-head screw at the clamping adapter.

4. Insert the sparger/dip tube into the clamping adapter from below.

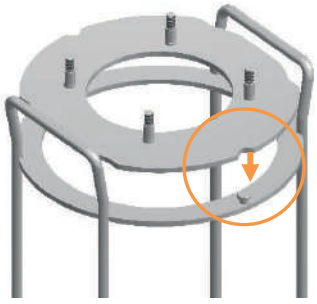


5. Set the desired mounting depth.
6. Tighten the slotted-head screw.

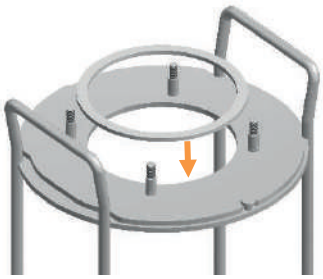
8.1.4 Inserting the Vessel into the Vessel Holder

To insert the glass vessel into the vessel holder, proceed as follows:

Procedure



1. Place the flange onto the ring of the vessel holder:
   the two opposing recesses on the flange fit with the bolt on the ring.



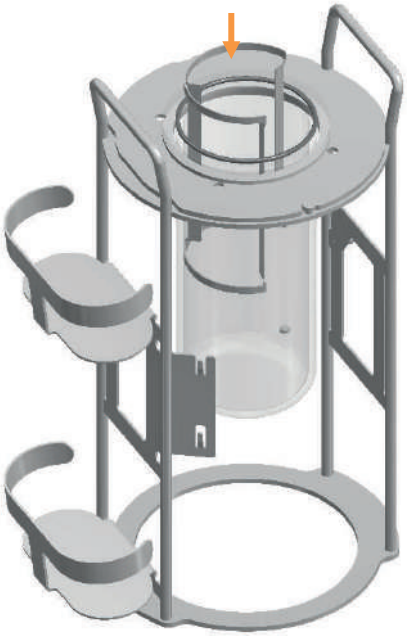
2. Place the damping ring onto the flange.

3. Insert the vessel carefully.

8.1.5 Inserting the Baffles

Proceed as follows:

Procedure



1. Insert the baffles carefully into the glass vessel.

8.1.6 Moistening/Filling the Culture Vessel

If the medium in the culture vessel is to be autoclaved, this can take place before the top plate is put in position and the additional component parts are mounted.

Note the following about filling the culture vessel before autoclaving:

- Before autoclaving, only top up with heat-resistant media.
- During autoclaving, evaporation may result in a loss of volume and thus to increased salt concentration in the medium. If necessary, top up with sterile water.

INFORMATION

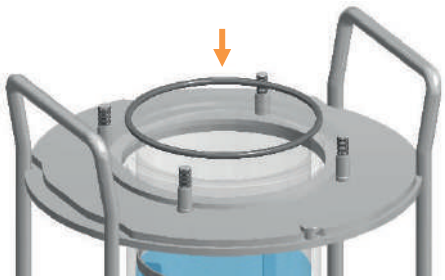
Development of steam is not possible when autoclaving an empty and dry culture vessel. Successful sterilisation is not guaranteed.

Ensure that there is liquid in the culture vessel (approx. 10 mL of water per litre of total volume).

8.1.7 Fitting the Vessel Top Plate

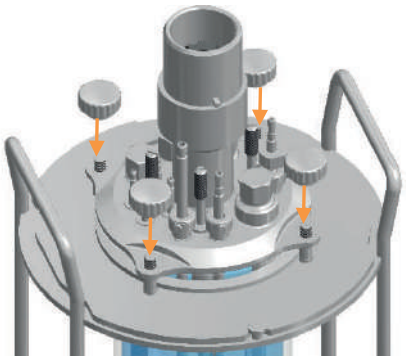
Proceed as follows to fit and fix the vessel top plate:

Procedure



1. Fit the O-ring for the top plate gasket into the groove on the edge of the vessel.

2. Place the top plate carefully and with the correct alignment into position and ensure that component parts do not touch the baffles.



3. Tighten the knurled screws on the top plate by hand (no tool!) crossways.

ATTENTION

If the screws are tightened too much, components may be damaged, which can result in failure of the equipment. The screws may not be tightened with a tool under any circumstances.

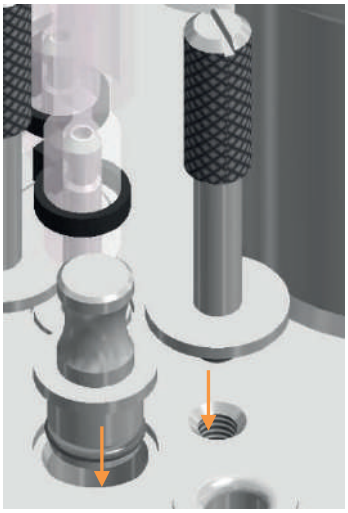
This applies to all screw connections where the instructions specify that they must be tightened by hand

8.1.8 Mounting the Blanking Plugs

For mounting the different blanking plugs, proceed as follows:

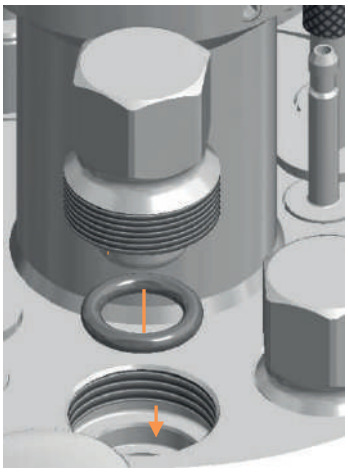
Ø 10 mm ports

Procedure



1. Insert the blanking plug with fixed O-ring into all unused ports.
2. Fix with a fastening screw.

Procedure



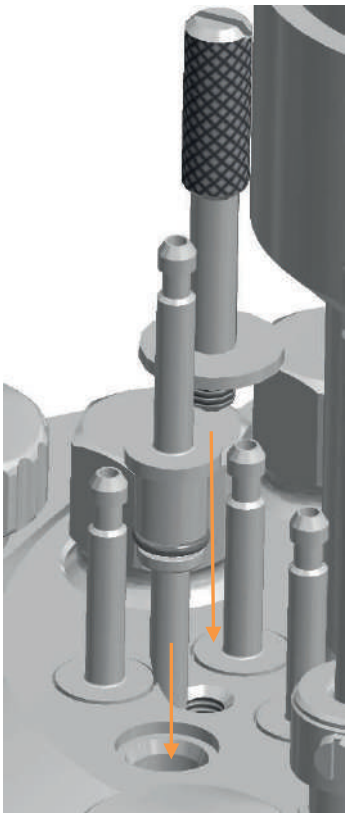
Ø 12 mm / Pg13.5 ports

1. Insert the O-ring and blanking plug into all unused ports.
2. Tighten by hand.
3. Use the hexagon socket spanner to make it hand-tight.

8.1.9 Mounting Addition Port Adapters

Proceed as follows:

Procedure



1. Insert the addition port adapters with a fixed O-ring into the four 7.5 mm ports.
2. Fix it with the fastening screw.

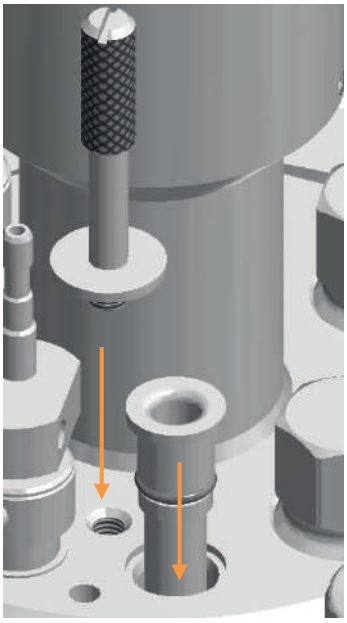
8.1.10 Mounting the Feed Needle(s)

The procedure for mounting one or more feed needle(s) instead of addition port adapters is the same as for the mounting of the addition port adapters. For details, see the chapter “Mounting Addition Port Adapters”.

8.1.11 Mounting the Pocket for Temperature Sensor (Pt100)

Proceed as follows:

Procedure



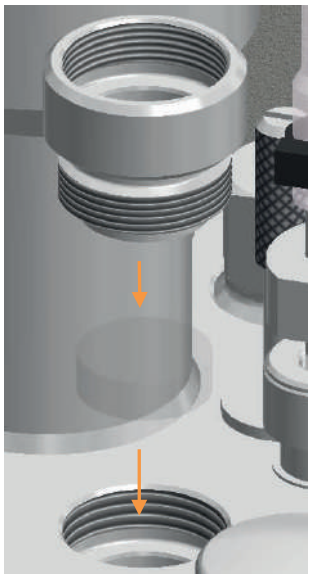
1. Insert the pocket with the fixed O-ring into the 10 mm port.
2. Fix it with the fastening screw.

8.1.12 Equipping the Port with a Septum Collar and Septum for Inoculation

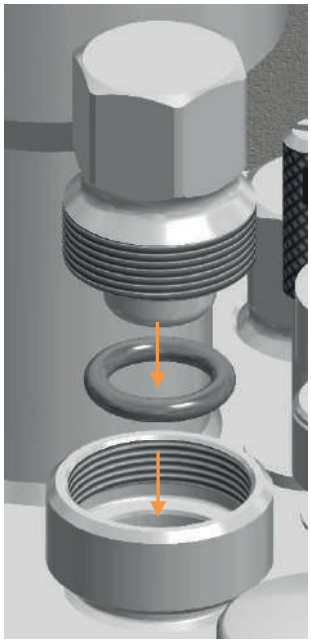
For later inoculation with a syringe, the 12 mm/Pg13.5 port in the top plate must be prepared as follows:

Procedure

1. Ensure that there is no O-ring in the port; if there is, remove it.



2. Insert the septum (inoculation diaphragm) into the port.
3. Screw the septum collar into the port by hand.



4. Insert the blanking plug equipped with an O-ring into the septum collar and screw it tight by hand.

   If necessary, use the hexagon socket spanner to make it hand-tight.

8.1.13 Preparing the Dip Tube/Addition Port Adapter for Inoculation

If later inoculation is to be carried out by means of a dip tube or addition port adapter, proceed as follows:

Procedure

1. Fit the dip tube with the clamping adapter or addition port adapter in the port.
2. Place a piece of silicon hose onto the dip tube/addition port adapter.

3. Equip the hose for a sterile hose connection. (Depending on the application: rapid coupling, sterile connector or weldable hose with sterile filter).
4. Secure the hose transition points with cable ties.

8.1.14 Mounting the Exit Gas Cooler

Mount the exit gas cooler as follows:

Procedure

1. Attach the O-ring to the thread of the exit gas cooler.
2. Screw the exit gas cooler onto the thread in the 12 mm/Pg13.5 port by hand.
3. Align the exit gas cooler to ensure that handling of other component parts is impaired as little as possible.
4. Check to ensure that the exit gas filter is fitted securely.
5. Cap the exit gas filter loosely with a little aluminium foil

INFORMATION

A humidifier bottle with antifoam reagent can be installed between exit gas cooler and the exit gas filter if significant foam formation is expected.

Take the following into account for autoclaving:

- Only use a new, clean and dry exit gas filter and fix it in such a way that it cannot slip.
- ALWAYS keep the exit gas line - hose at the exit gas cooler with secured exit gas filter - open.

CAUTION

If pressure equalisation does not take place via a top plate opening or the mounted exit gas cooler, overpressure or vacuum in the culture vessel may occur during autoclaving.

8.1.15 Preparing the Sensors

All sensors that come into contact with the medium are mounted before autoclaving and are sterilised together with the culture vessel.

Note the following about all sensors:

- Mount all sensors by hand – do not use any tools!
- Mount the sensors in such a way that they cannot come in contact with other components or the glass vessel.
- If the mounting depth of is adjustable (mounting with electrode holder/clamping adaptor), make sure the mounting depth is set correctly prior to autoclaving, as later adjustment represents a contamination risk.

pH sensor

- Calibrate the pH sensor on the operating panel before mounting and autoclaving.

pO_2 sensor

- Mount the pO_2 sensor in such a way that it has good access to the flow and there is no risk of bubbles collecting. (Calibration is carried out AFTER autoclaving)

pH sensor & pO_2 sensor

- For vessels with a nominal width of 90 and 145: screw the sensors directly into 12 mm/Pg13.5 port.
- For vessels with a nominal width of 115: mount the sensors with an electrode holder.

! ATTENTION

Risk of damage to the pH and pO_2 sensors. Covering the sensor heads with aluminium foil during autoclaving may lead to water gathering under the film, thus damage the contacts on the sensor head.

pH and pO_2 sensor heads may **NOT** be covered with aluminium foil during autoclaving!

For details on the safety, technical data, usage and maintenance requirements for the pH and pO_2 sensors, see the separate documentation provided by the sensor manufacturers.

8.1.16 Calibrating the pH Sensor

For a reliable pH measurement, a 2-point calibration with an upper and lower reference buffer must be carried out before each cultivation. The pH sensor must be calibrated before autoclaving. This is carried out on the operating panel.

Procedure

1. Connect the sensor cable. (For more details, see the chapter “Connecting the pH Sensor”).
2. Switch on the equipment using the main switch.

   The operating panel is switched on automatically and the system is started.
3. Calibrate the pH sensor in accordance with the detailed description in the main chapter “Operation”, chapter “Calibrating the pH Sensor”.

INFORMATION

If the pH sensor has already been calibrated before connection to the system, the bioreactor will use this data and calibration using the operating panel is no longer necessary.

8.1.17 Mounting a Sensor into a 12 mm Port

For culture vessels with nominal widths of 90 and 145, sensors can be screwed directly into 12 mm/Pg13.5 ports. To do so, proceed as follows:

Procedure

1. Slide the O-ring onto the sensor.

2. Insert the sensor into the port.
3. Screw the sensor on its thread into the port by hand.

8.1.18 Mounting Sensors with Electrode Holder

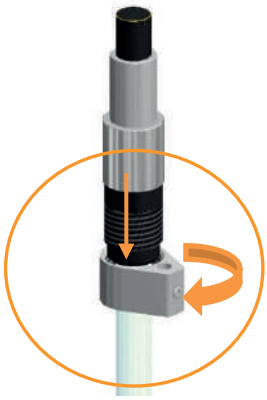
For the mounting of a sensor in a 12 mm/Pg13.5 port for culture vessels with a nominal width of 115, an electrode holder must be used.

To do so, proceed as follows:

Procedure

1. On the electrode holder, lightly loosen the grub screw in the support guide with the key.

2. Pull the support guide from the guide bar.

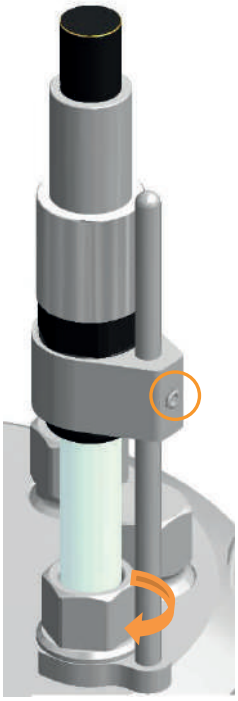


3. Insert the sensor into the support guide and tighten it.

Before Cultivation

4. Insert the sensor into the hollow screw with the thread pointing in the downward direction.
5. Fit the fork of the guide bar into the groove of the hollow screw.
6. Push the hollow screw and the guide bar together upwards and insert the guide bar into the hole of the support guide.

7. Slide the O-ring onto the sensor and insert the sensor into the port.
8. Adjust the sensor to the desired height.



9. Screw the sensor on the hollow screw into the port and tighten it.
10. Tighten the grub screw in the support guide with the key.

8.1.19 Mounting the Antifoam Sensor

Please note the following points for mounting:

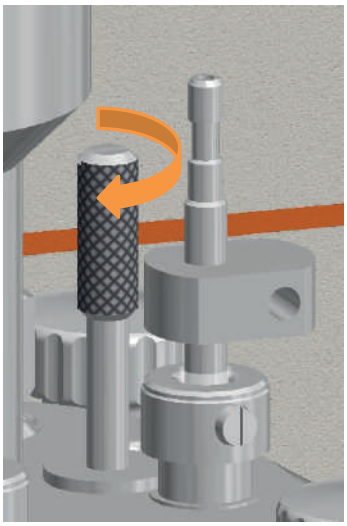
- The antifoam sensor is equipped with transparent insulation that must be intact, as otherwise a continuous signal “Foam/liquid detected” may be generated.
- The sensor head must not touch the clamping adaptor, otherwise a continuous short-circuit is generated, indicating “Foam/liquid detected”.
- The clamping adapter on the sensor must be equipped with an intact O-ring.

Proceed as follows for mounting:

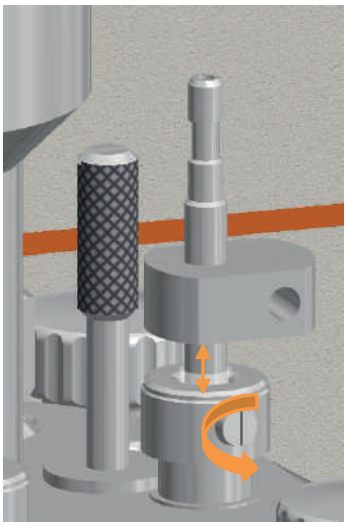
Procedure

1. Remove the protective cap from the sensor.
2. Insert the sensor into the port.

Before Cultivation



3. Fix the clamping adapter with the fastening screw.



4. Loosen the slotted-head screw at the clamping adaptor.
5. Set the desired mounting depth of the sensor carefully.

6. Tighten the slotted-head screw carefully.

ATTENTION

If the sensor is fixed too tightly in the clamping adapter, or the mounting depth of the sensor is changed while the screw on the clamping adapter is tightened, the sensor insulation may be damaged.

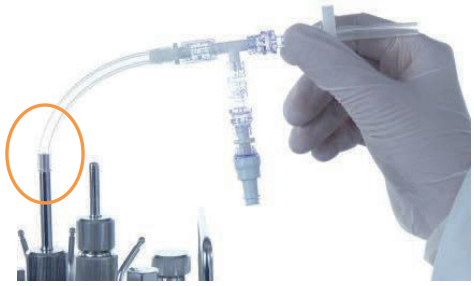
8.1.20 Preparing the Super Safe Sampler

INFORMATION

The following figures are for general purposes of comprehension.

In order to prepare the Super Safe Sampler sampling system for autoclaving, proceed as follows:

Procedure



1. Attach the hose of the valve group on the dip tube.

2. Secure the hose with a cable tie.

3. Tighten the sample valve carefully by hand in a clockwise direction.

   This ensures that the non-return valve/sample valve screw connection is tight.

4. Turn the sterile filter carefully by hand in a clockwise direction.

   This ensures that the non-return valve/sterile filter screw connection is tight.

5. Cover the valve group loosely with aluminium foil.

6. Clamp off the hose on the dip tube.

8.1.21 Mounting the Sparger Hose and the Inlet Air Filter

The sparger must be equipped with the hose and inlet air filter before autoclaving.

To do so, proceed as follows:

Procedure

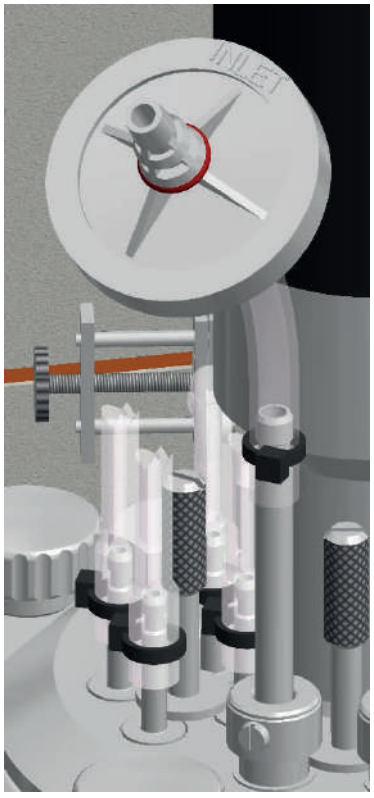
1. Cut a short piece of hose:

   - 1.5 L culture vessels: silicone hose Ø = 2 x 6 mm
   - 3.0 L and 6.0 L culture vessels: pressure hose, transparent, Ø = 5 x 10 mm.

2. Fit the inlet air filter to the hose piece:

   - 1.5 L culture vessel: filter with red marking, Ø = 37 mm, fit it in the direction of the air flow to the hose end. The nozzle with the red *INLET* marking remains exposed.

- 3.0 L and 6.0 L culture vessels: filter without marking, Ø = 50 mm, fit it in either direction to the hose end.



3. Fit the hose to the sparger.

   The figure to the left shows an inlet air filter for 1.5 L culture vessels as an example.

4. Secure the ends of the hose with the cable tie.
5. Clamp off the hose with a hose clamp.
6. Lightly cap the inlet air filter with aluminium foil.

8.1.22 Preparing the Reagent Bottles, Pumps and Hoses

250 mL reagent bottles are supplied as standard in the equipment package. These fit in the reagent bottle holder that is built into the vessel holder. Before delivery with the equipment, the reagent bottles are equipped with filters for pressure equalisation and hoses of the correct length, and connected to the pump heads.

ATTENTION

Damaged hoses and/or clogged sterile filter may lead to undesired pressure conditions in the reagent bottles.

– Ensure each reagent bottle is equipped with an open pressure equalisation line with a clean and dry filter.
– Only use clean, intact hoses and they are firmly attached.

Before Cultivation

Below is a detailed description of how reagent bottles are equipped properly and connected to the pumps and culture vessel.

Connecting the reagent bottles to the pumps and culture vessel

To connect the reagent bottles with the pumps and the culture vessel, proceed as follows:

Procedure

1. Cut two long silicone hoses (Ø = 2 x 6 mm) per pump/reagent bottle.

INFORMATION

The length of the silicone hoses must be selected to ensure that the hose connections between the reagent bottles, pumps and culture vessel do not have any tensions or kinks.

2. Thoroughly rinse the silicone hoses with distilled water.

3. Connect the silicone hoses and pump hoses of the pump heads with hose connectors.

   For the **Fill** function:

   - Right-hand side = suction side = hose line to reagent bottle.
   - Left-hand side = pumping side = hose line to culture vessel.

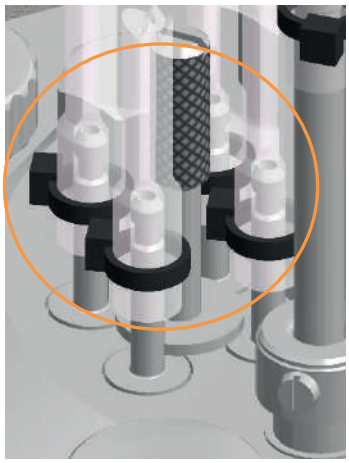
     See arrow for direction of rotation.

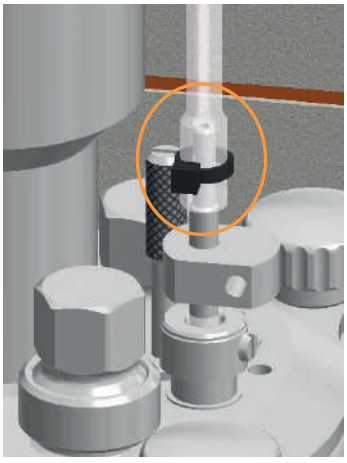
4. Secure with cable ties.

Connection between pumps and culture vessel

Procedure

1. Fit silicone hoses for base, acid and feed to addition port adapter and/or feed needle(s) and secure them with cable ties.





2. Attach the silicone hose of the antifoam pump to the mounted antifoam sensor in the culture vessel and secure it with a cable tie.

Connection between reagent bottles and pumps

Procedure

1. Ensure that a hose is fitted inside the reagent bottles at the exposed hose connection (without sterile filter); fit one if not:
   a) the end of the hose does not touch the bottom of the bottle, otherwise the hose may get sucked against the bottom and no longer be able to pump liquid.
   b) the end of the hose is cut diagonally. In this case the hose end can touch the bottom of the bottle.
2. Label the reagent bottles in accordance with their content.
3. Depending on the application: Fill the reagent bottles with reagents and reclose them with their lid.

ATTENTION

Usage of the highly corrosive hydrochloric acid HCl as reagent leads to damage to components made of stainless steel such as e.g. component parts or the top plate.

Use only non-corrosive acids, e.g. phosphoric acid, instead.

INFORMATION

Fill reagent bottles with heat-resistant reagents only. Sterilise non-heat-resistant feed solution separately and only transfer it to the reagent bottle after sterilising.

4. Place the reagent bottles in reagent bottle and pump holders.

5. Attach the correct silicone hoses to available hose connections of each reagent bottle and secure them with cable ties.

6. Close silicone hoses with clamps as close as possible to the hose connections of the reagent bottles to ensure that no reagent can flow into the culture vessel.
7. Ensure that:
   - each reagent bottle is connected with the appropriate pump according to its contents. (Base to base pump, etc.)
   - filters are clean and dry; short hose line is open.
8. Cap the filter loosely with aluminium foil.

8.1.23 Sterile Hose Connections

If additional vessels are needed and these can only be connected to the culture vessel after autoclaving, such as vessels for the inoculum or bottles for sampling etc., rapid couplings (male/female), sterile connectors or – if weldable hoses are used – a hose welding device can be used to form a sterile connection.

The connection pieces must be fitted to the appropriate hoses before autoclaving. Rapid couplings are connected after autoclaving in a sterile workbench. Sterile connectors and hose welding devices allow sterile connecting without a sterile workbench.

8.1.24 Setting the Pumps

If the pumps are not used with the default settings, we recommend that the appropriate settings are now made on the operating panel.

It is possible, for example, to estimate and display the volume (in mL) that has been pumped since the Batch (process) started. To this end, the diameter of the hose used must be selected.

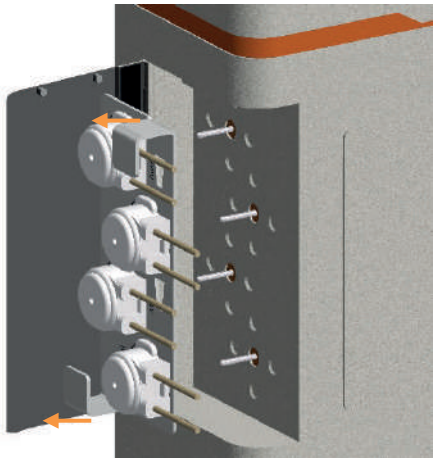
For details on the pumps and the setting options, see the main chapter “Operation”, the “PUMPS parameter group” chapter and the associated sub-chapters.

8.1.25 Removing the Pump Heads

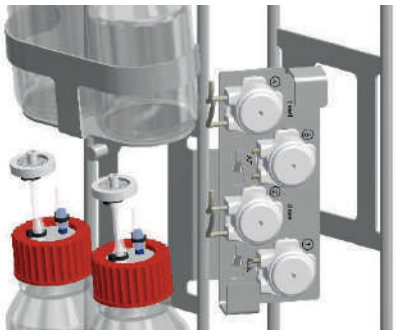
To remove the pump heads from the basic unit, proceed as follows:

Procedure

1. Swing open the pump cover.



2. Remove the mounting plate with the pump heads from the drive shafts by holding the two handles.



3. Place the mounting plate with the pump heads onto the pump holder on the vessel holder.

8.1.26 Fitting the Cone Plug for Drive Hub

In order to prevent the penetration of condensation water into the dive hub during autoclaving, the cone plug provided in the starter set must be fitted.

ATTENTION

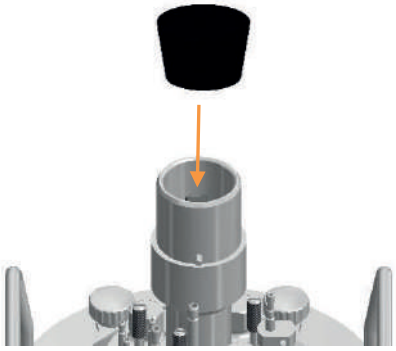
Risk of loss of property du penetration of condensation water into the drive hub!

Always autoclave the culture vessel with the cone plug fitted to the drive hub!

Proceed as follows:

Procedure

1. Plug the cone plug into the opening of the drive hub.



8.1.27 Checklist Before Autoclaving

Check and ensure the following items before autoclaving:

Culture vessel
All necessary O-rings are fitted.
All unused ports are closed with blanking plugs
Connection for inoculation is equipped with septum, septum collar and blanking plug
Drive hub is equipped with **cone plug**.
There is liquid in the culture vessel (autoclavable medium or approx. 10 mL water per litre working volume).

Reagent bottles, hoses and pumps
Reagent bottles are exclusively filled with autoclavable reagents, correctly labelled and connected with the culture vessel and the pump heads via hoses.
Reagent bottles are equipped with filters for pressure equalisation

Reagent bottles are placed in reagent bottle holders and pump heads are placed on the pump holder with a mounting plate.

Super Safe Sampler
The valve group is connected to the dip tube in the culture vessel by means of a hose.
The valve group is lightly capped with aluminium foil.

Sparger & exit gas cooler
The sparger is equipped with a hose and an inlet air filter.
The exit gas cooler is equipped with a new securely fastened exit gas filter.

Filters & hoses
All filters are clean, dry and lightly capped with aluminium foil.
There are no open hose ends.
All hose transition points are secured with an autoclavable cable tie or hose clamp to prevent them from slipping.
Hoses on the reagent bottles, for sampling and the gassing system (sparger) are clamped off with hose clamps.
The exit gas hose is **NOT** clamped off.
The hoses are undamaged; the hose lines show no kinks and are not able to kink.

Sensors
All sensors required are mounted and, if necessary, calibrated.
The antifoam sensor is mounted, set for the correct mounting depth and connected to the correct reagent bottle.
The temperature sensor of the autoclave is inserted into the pocket for the temperature sensor of the culture vessel.
Do not cover the pH and pO_2 sensors with aluminium foil!

8.1.28 Autoclaving

Before cultivation starts, the culture vessel is autoclaved in accordance with the application in question. The culture vessel can be autoclaved with or without medium.

Adhere to the following:

- Never autoclave the culture vessel dry; see also the chapter “Moistening/Filling the Culture Vessel”.

Before Cultivation

INFORMATION

Development of steam is not possible when autoclaving a completely empty and dry culture vessel. Successful sterilisation is not guaranteed.

Ensure that there is liquid in the culture vessel (approx. 10 mL of water per litre of total volume).

- If necessary, pump off any remaining water after autoclaving by means of the dip tube.
- Sterilise all liquid, heat-instable components separately and add them after autoclaving.
- If the medium is autoclaved in the culture vessel, you may then need to add sterile water to make up the volume.

When transporting the culture vessel to/from the autoclave, note the following:

- Always transport the culture vessel in the vessel holder.
- Always transport the culture vessel to/from the autoclave in pairs and use suitable auxiliary equipment when transporting the culture vessel.

WARNING

Depending on the design, accessories and fill level, the culture vessel may be too heavy to be carried by one person alone.

Proceed as follows to autoclave the culture vessel:

Procedure

1. Place the culture vessel into the autoclave.
2. Ensure that the culture vessel and the accessories do not touch the inner wall of the autoclave.
3. Ensure that the exit gas filter is open.
4. Insert the temperature sensor of the autoclave into the pocket for the temperature sensor.
5. Select the program for liquids.
6. Autoclave the culture vessel in accordance with the operating manual of the autoclave manufacturer.

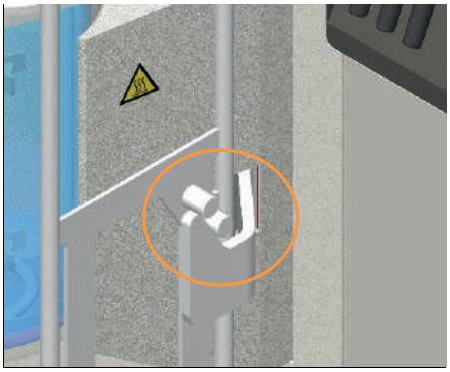
8.2 Connecting the Culture Vessel and Preparing the Cultivation

As soon as the culture vessel with the accessories has cooled sufficiently, it can be hung up within the basic unit and the various cable and tube connections between the basic unit and the culture vessel can be established.

8.2.1 Hang the Culture Vessel in Place and Fit the Pump Heads

Proceed as follows:

Procedure



1. Hang the vessel holder into place on the two hooks on the thermal block adapter.
2. Pull off the mounting plate with the pump heads from the pump holder
3. If necessary, flip up the pump cover plate.

4. Plug the mounting plate with the pump heads onto the pump motor drive shafts and close the cover plate.

8.2.2 Filling the Reagent Hoses

In order to prepare the reagent hoses for operation, you must use the **Fill** function on the operating panel to fill up with reagent via the correct pump.

Before filling, remove the clamps from the reagent hoses.

WARNING

When using heavily corrosive reagents (acids and bases), it is particularly important only to use suitable and undamaged hoses. They must also be securely fastened.
Furthermore, the exit gas filter must not be blocked. This ensures that no pressure builds up and no reagent escapes due to burst hoses.

When filling, ensure that no reagent escapes into the culture vessel, if possible.

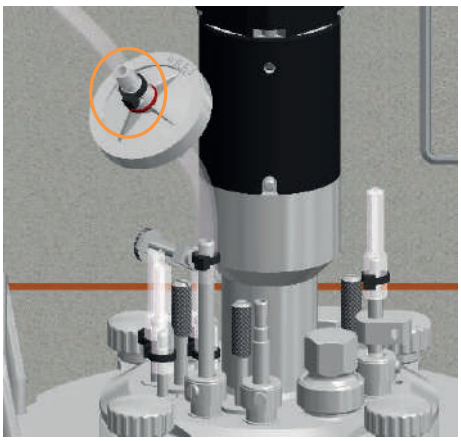
For details on filling, see the main chapter “Operation”, chapter “Parameter Group PUMPS”.

8.2.3 Connecting the Gassing

To connect the sparger to the gassing, proceed as follows:

Procedure

1. Remove the aluminium foil from the inlet air filter.



2. Insert the gassing hose of the basic unit to the inlet air filter of the sparger and secure it in place with a cable tie.

   The figure to the left shows an inlet air filter for 1.5 lit. culture vessels as an example.

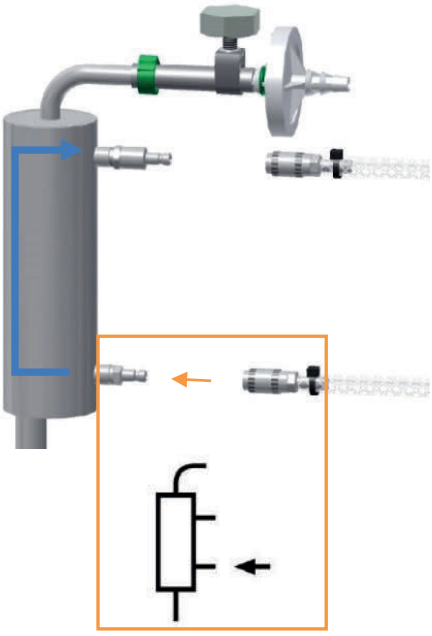
3. Remove the hose clamp.

8.2.4 Connecting the Exit Gas Cooler

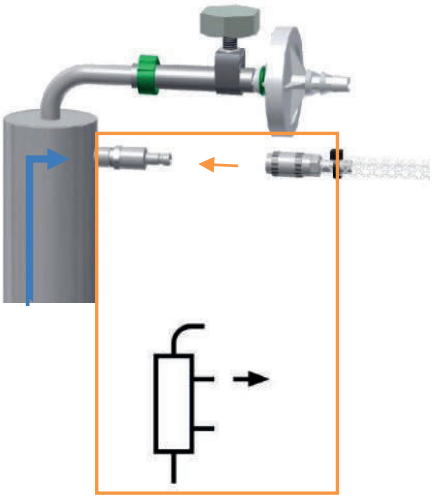
To connect the exit gas cooler to the basic unit, proceed as follows:

Procedure

1. Remove the aluminium foil from the exit gas filter.



2. Insert the rapid coupling of the water **inlet** hose – note the symbol on the basic unit - onto the **lower** connection nozzle on the exit gas cooler.



3. Insert the rapid coupling of the water **outlet** hose – note the symbol on the basic unit - onto the **upper** connection nozzle on the exit gas cooler.

INFORMATION

The exit gas cooler only works when the temperature control system is switched on (*Temperature* ON parameter)

8.2.5 Coupling the Motor

For routine operation, it is not necessary to plug in and unplug the motor cable. The motor connected during installation is only coupled before cultivation. For details about connecting the motor cable, see the main chapter “Installation and Initial Operation” and the chapter “Connecting the Motor Cable”.

To couple the motor, proceed as follows:

Procedure

1. Place the motor onto the drive hub with the groove aligned with the pin on the drive hub.

   The motor is held in its position.

8.2.6 Filling the Culture Vessel

Depending on the application, the vessel can be filled after autoclaving. To prevent foam formation during filling, add the medium via a dip tube.

To do so, proceed as follows:

Procedure

1. Sterilise the medium separately.
2. If necessary, pump off any water that remains in the culture vessel.
3. Establish a sterile hose connection between the culture vessel and the medium container.
4. Pump the desired quantity of medium into the culture vessel.
5. Clamp off the medium hose; if necessary, apply a welded seal.
6. Disconnect the medium container from the culture vessel; if necessary, retain it as a harvest or waste container.

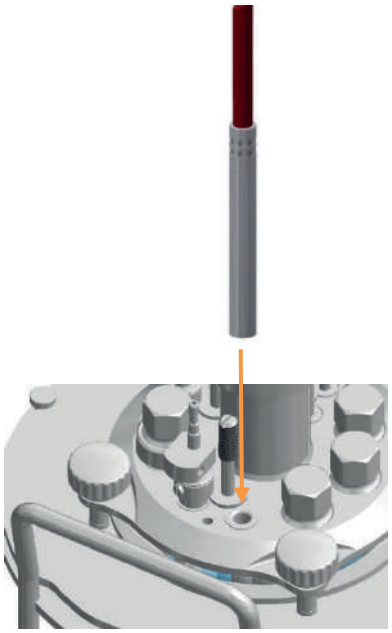
INFORMATION

If the stirrer is turning on the surface of the medium, foam will be formed. For this reason, only switch on the stirrer if it is fully covered by medium.

8.2.7 Connecting the Temperature Sensor (Pt100)

The temperature sensor is not in direct contact with the medium.

Procedure

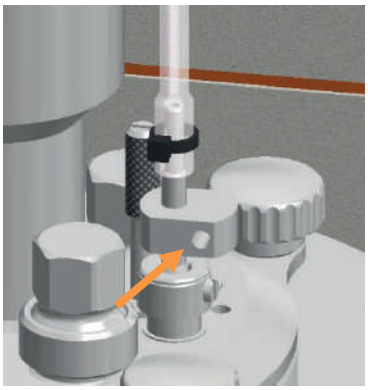


1. Insert the sensor into the pocket in the vessel top plate as far as it will go..

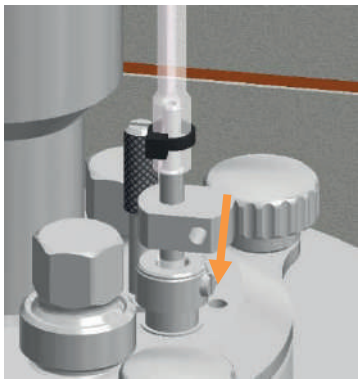
8.2.8 Connecting the Antifoam Sensor

To connect the antifoam sensor, the two banana connectors of the sensor cable must be inserted as follows:

Procedure



1. Insert the red banana plug into the connector on the sensor head.



2. Insert the black banana plug into the earth connection in the top plate.

8.2.9 Connecting the pH Sensor

To connect the pH sensor, proceed as follows:

Procedure

1. Remove the red protective cap from the sensor cable.

2. Place the connector of the sensor cable onto the sensor head and screw into place.

   Ensure the cable is not twisted or buckled.

8.2.10 Connecting the pO_2 Sensor

To connect the pO_2 sensor, proceed in the same manner as with the pH sensor; see the chapter “Connecting the pH Sensor”.

8.2.11 Calibrating the pO_2 Sensor

Generally speaking, the following applies: The pO_2 sensor should be calibrated after autoclaving has been performed because the sterilisation process may change the steepness of the pO_2 sensor. As a rule, a 1-point calibration to 100 % is usually sufficient for exact measurement, and should be carried out before each cultivation.

Depending on the specifications defined by the user, the pO_2 sensor is calibrated either before the vessel is filled with medium or afterwards, in the prepared medium.

For more details on the calibration refer to main chapter “Operation”, chapter “Calibrating the pO_2 Sensor”.

8.2.12 Checking the Hoses and Hose Connections

Check and ensure the following items before each cultivation:

- Hoses show no kinks and are not able to kink.
- Hoses are undamaged and show no weaknesses.
- Gas hoses and connections do not show any leaks.
- Hose lines are as short as possible.
- Hoses are secured with cable ties and/or hose clamps.
- Only the pressure hoses supplied by the equipment manufacturer are connected as supply lines (water, gas) between the in-house connections and the equipment.

9 Cultivation

The following sections describe the work necessary for the performance of and after the completion of a cultivation, before the culture vessel with accessories is thoroughly cleaned and then prepared for another cultivation.

This essentially comprises:

- Preparing the medium (starting a Batch)
- Sampling
- Inoculation
- Harvest
- (Stopping the Batch), if necessary emptying the vessel
- Autoclaving the culture vessel and accessories

The requirement for the first item is that the culture vessel and accessories are autoclaved, cooled and connected to the basic unit. All cable and hose connections between the equipment and the culture vessel, including the reagent bottles, are present, pump heads are mounted and the reagent hoses are filled. Depending on the user specifications, the pO_2 sensor is already calibrated.

9.1 Preparing the Medium

Before the first sampling, which usually takes place as a ‘zero sample’ before inoculation, and before the inoculation itself, the medium must be warmed to the desired temperature. If necessary, the pO_2 concentration and the pH are set. The time required for this depends on the working volume.

Set and activate the desired setpoint of the parameter in question on the operating panel, and start the Batch (process).

⚠ CAUTION

If pressure equalisation does not take place via a top plate opening or the mounted exit gas cooler, overpressure in the culture vessel may occur during cultivation as a result of warming, gassing or fermentation processes.

– Exit gas line - hose at the exit gas cooler with secured exit gas filter - ALWAYS keep open.

– Only use clean and dry exit gas filters.

For details on operation, see the corresponding chapter in the main chapter “Operation”.

INFORMATION

Depending on the specifications defined by the user, the pO_2 sensor is calibrated either before the vessel is filled with medium or afterwards, in the prepared medium.

For more details on the calibration refer to main chapter “Operation”, chapter “Calibrating the pO_2 Sensor”.

9.2 Sampling

Samples are taken from the culture vessel to gain material for offline analysis.

INFORMATION

Using **SAMPLE NOW** on the operating panel, the sampling can be logged in the electronic logbook and assigned a sample ID. For more details, see main chapter “Operation”.

The method of sampling can vary due to the different analyses carried out by the operator.

The sampling procedure using the standard sampling system, Super Safe Sampler, is described below.

Before starting, observe the following:

WARNING

Culture solution could emerge from the vessel if the sample valve mechanically fails. This could lead to serious health risks in the event of applications with pathogenic organisms.

– When working with pathogenic organisms, always additionally clamp off the sampling hose with a metal (!) clamp.
– Only remove the clamp when sampling.
– Reattach the clamp before removing the syringe from the sample valve.

Cultivation

WARNING

Loose screws at components could lead to the penetration of unsterile air or contamination of the environment.

Before and after autoclaving: Check that all screws are tightly screwed in and, if necessary, tighten them manually

If the sample is to be further aseptically processed, use a sterile syringe and sterile closing caps.

For details, see the main chapter “Accessories”, chapter “Sampling System Super Safe Sampler”, section “Aseptic Sampling”.

Proceed as follows:

Procedure

1. Check that all screw connections of the valve group are tightly screwed in. If necessary, gently tighten the screw connections with two fingers.
2. Remove the clamp from the sampling hose.
3. If present: Remove the closing caps.
4. If desired: Disinfect the sample valve.
5. Screw open the Luer-Lock syringe on the sample valve.

6. Pull back the syringe plunger to remove the desired sampling volume.

If the dip tube was rinsed with air, air is sucked in first. Remove it as follows:

a) Unscrew the syringe from the valve.
b) Hold the syringe with the plunger downwards so that the medium remains in the syringe.
c) Push the air out of the syringe.
d. Screw the syringe onto the sample valve.
e) Draw in again.

7. Attach the clamp to the sampling hose.

Rinsing the dip tube with sterile air

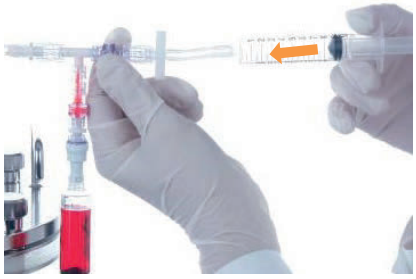
The dip tube and its sampling hose can be filled with sterile air after taking a sample.

INFORMATION

Only use a clean and dry syringe to avoid blocking the sterile filter. This syringe can be reused as often as desired, since air is provided via a sterile filter.

To do so, proceed as follows:

Procedure:



1. Insert the syringe onto the hose at the sterile filter and push air through.

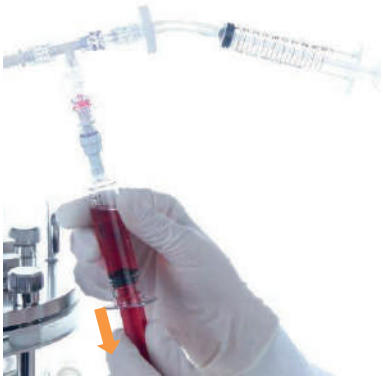
   The remaining liquid in the hose and in the dip tube is pushed back into the vessel.

2. Remove the syringe from the sterile filter to fill it with air again.
3. Repeat steps 1 and 2 as many times as necessary until bubbles rise out of the dip tube.

Removing residual fluid

To remove residual fluid from the system, proceed as follows:

Procedure:



1. Hold the syringe with sample downwards, pull back the plunger.

   This removes all but a few µL of the residual fluid.

2. Hold the sample valve with one hand; unscrew the syringe with the other.

3. If desired: Place the closing caps on the sample valve and on the syringe with the sample.

9.3 Inoculation

Check and ensure the following items before inoculation:

- Medium has been filled.
- Heat-labile, separately sterilised substances are present.
- The reagent bottles are connected with the pumps and the culture vessel, and are filled with a sufficient amount of reagents and feed solution for the duration of the entire cultivation process.
- The hoses of the reagent bottles are filled.
- The correct operating temperature has been reached.
- The required stirring speed is set.
- The sensors are calibrated and the control is correct (or not yet switched on).

- All clamps have been removed (except for sampling system).
- Utensils for the inoculation and vessels with inoculum are ready.

Methods

There are a number of ways to add medium or inoculum before and during cultivation:

- In a small volume, with the syringe via the septum
- Via the addition port adapter from the reagent bottle (a sterile hose connection is required for this method).
- Via the dip tube from the reagent bottle (a sterile hose connection is required for this method).

These methods are described below.

The implements for inoculation with a syringe are standard accessories for the equipment. This inoculation method is particularly suitable for all vessel sizes of the equipment.

9.3.1 Inoculation with a Syringe

Proceed as follows for the inoculation:

Procedure

1. Fill the syringe with the required amount of inoculum.
2. Unscrew the blanking plug from the septum collar.

   As a possible additional protection against contamination:

   Before piercing, drop a few drops of ethanol (70 %) on the septum.
3. Pierce the septum and inject the inoculum.
4. Remove the needle from the septum and close the septum collar with a blanking plug.

9.3.2 Inoculation Using Dip Tube / Addition Port Adapter

Proceed as follows during inoculation:

Procedure

1. Fill the inoculum under sterile conditions into the prepared container.
2. Create a sterile hose connection with the dip tube/addition port adapter.
3. Transfer the desired volume of inoculum into the culture vessel. Pump it, if necessary.
4. Clamp off the hose with the hose clamp, weld it if necessary.

9.4 Harvest

The culture can be harvested at the end of the cultivation. To prevent possible sedimentation from the culture, the stirrer can be switched on during harvesting. If necessary, activate gassing for sensitive cultures. However, all other parameters should be switched off, provided there are no other specifications for the user.

The following possibilities exist for the harvest:

a) Transfer to another vessel

To transfer the contents of the vessel to another container in a laminar flow cabinet.

b) Pump-down via a sterile hose connection

To do so, proceed as follows:

Procedure

1. Make a sterile connection between the hose at the dip tube for harvest and the new vessel.
2. Connect the hose to one of the pumps on the equipment or to an external pump.
3. Pump the desired amount of culture into the new vessel.

INFORMATION

Only switch on the stirrer if it is fully covered by medium, as foam otherwise forms.

4. Switch off all parameters at the operating panel and stop the Batch (process) at the operating panel.

INFORMATION

Always stop the running Batch (process) on the operating panel. If it is stopped by pressing the main switch, it is akin to a power interruption. This means that when it is switched on again, the previous settings are adopted but control of the parameters remains switched off.

9.5 Emptying the Culture Vessel

Depending on the user specifications, the culture vessel can be emptied either before or after autoclaving.

A previously emptied and culture vessel filled only with water for autoclaving is easier to clean afterwards.

For emptying the culture vessel, the same options as for harvesting are available. For more information, see the “Harvest” section.

If the culture will not be used further, it must be inactivated according to the current in-house instructions (e.g. by autoclaving or by lowering the pH value), and subsequently disposed of in an environmentally sound manner according to the local regulations.

9.6 Emptying the Reagent Hoses

Before autoclaving the culture vessel with accessories, all reagent hoses must be completely emptied using the corresponding pump. This can either be done manually or time-controlled at the operating panel.

It is also recommended to thoroughly rinse the hoses with water after emptying and before autoclaving.

INFORMATION

When doing so, be aware that if the vessel is not emptied and **feed needle(s)** are used instead of addition port adapters, when the hoses are emptied the vessel contents are simultaneously pumped back into the reagent bottle.

9.7 Switching off the Equipment

When the harvest is finished or the culture vessel has been emptied and the reagent hoses are also empty, the equipment can be switched off.

Proceed as follows:

Procedure

1. Ensure that the Batch (process) has been stopped. If necessary, stop it using **Stop Batch.**

INFORMATION

Always stop the running Batch (process) on the operating panel. If it is stopped by pressing the main switch, it is akin to a power interruption. This means that when it is switched on again, the previous settings are adopted but control of the parameters remains switched off

2. Switch off the equipment at the main switch.
3. Let the motor cool down.

CAUTION

Risk of minor burns if the motor, which heats up during operation, is touched!

4. Close the supply lines (water, gas).
5. Autoclave the vessel, component parts and accessories as per the user-specific specifications and then clean them.

9.8 Autoclaving the Culture Vessel After Cultivation

After emptying the culture vessel and before cleaning, the culture vessel must be autoclaved with all accessories.

When doing so, do not autoclave the culture vessel when completely dry and observe the same safety regulations as when autoclaving before cultivation.

Before starting, ensure:

- There is liquid in the culture vessel (autoclavable medium or approx. 10 mL water per litre working volume).
- Reagents and feed solution have been pumped back out of the hoses.
- The equipment is switched off.
- The motor has cooled down.

Proceed as follows to prepare the culture vessel and accessories for autoclaving after cultivation:

Procedure

1. Clamp off the hoses of the reagent bottles.
2. Clamp off the hose of the sparger.

3. Remove all cable and hose connections between the basic unit and the culture vessel:
   a) Uncouple the motor and place it to the side.
   b) Unplug the sensor cables.
   c) Pull the temperature sensor out of the pocket.
   d) Disconnect the water inlet and water outlet hoses from the exit gas cooler.
   e) Remove the gassing hose (emerging from basic unit) from the inlet air filter on the sparger.

4. Lightly cover all filters with aluminium foil.

ATTENTION

Do not cover the pH and pO_2 sensors with aluminium foil!

5. Fit the cone plug into the opening of the drive hub.

ATTENTION

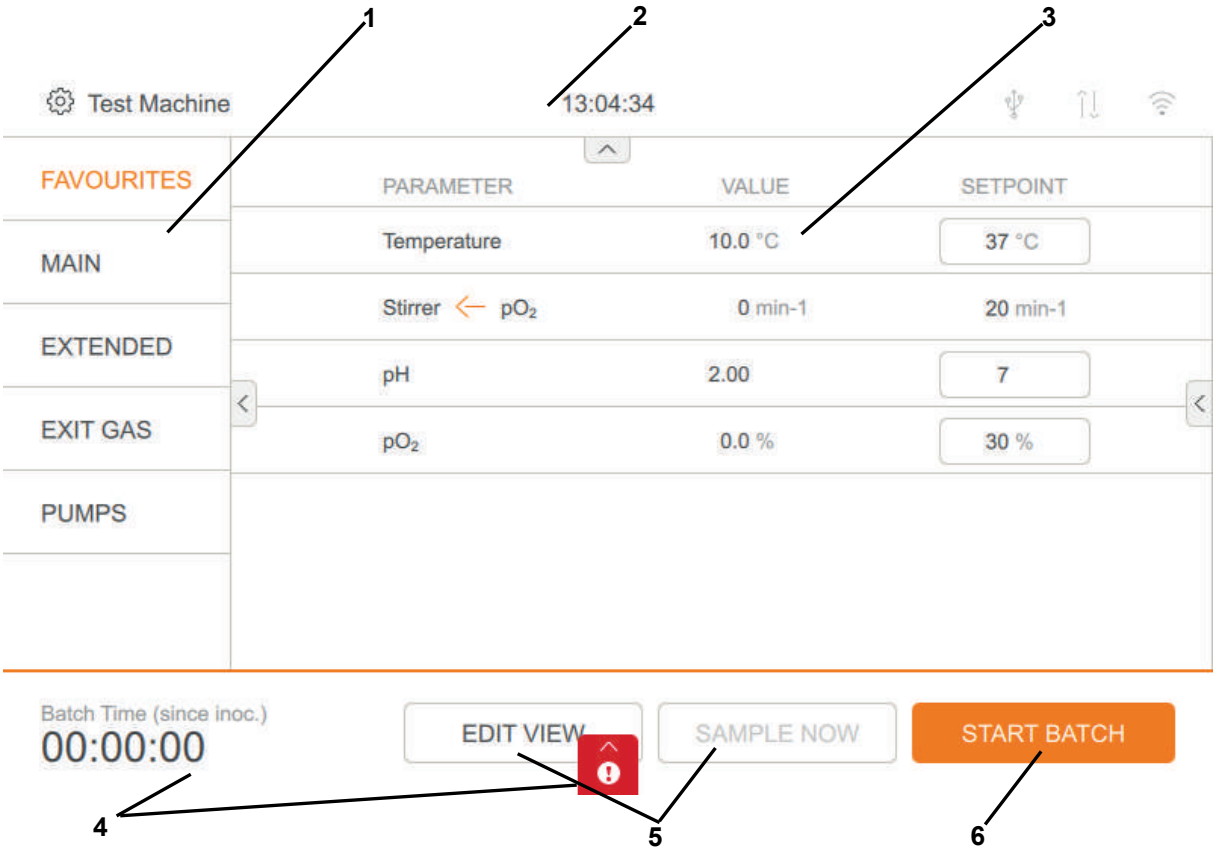
Risk of loss of property du penetration of condensation water into the drive hub!

Always autoclave the culture vessel with the cone plug fitted to the drive hub!

6. Open the pump cover.
7. Remove the mounting plate with pump heads from the drive shafts on the basic unit and place on the pump holder.
8. Check and ensure that the exit gas filter is free and dry and the exit gas hose is **OPEN**.
9. Insert the temperature sensor of the autoclave into the pocket on the culture vessel and autoclave the culture vessel.

10 Operation

10.1 Screen Areas, Menu Navigation and Control Elements



1 Left side with selection menus for system settings or parameter groups

2 Header with status displays

3 Main screen

4 Display of the Batch time (since inoculation) and possible alarms

5 Buttons for the selection of the parameter display and time stamp for sampling

6 Buttons with changing function for Batch start, inoculation time stamp and Batch stop

Header status display

The following symbols and displays are in the header:

- *Settings* symbol: to switch between menu selection for system settings and parameter groups

- Display of the current time

- Display for connected USB stick

- Display for an active connection to SCADA software

- Display for an active wireless connection

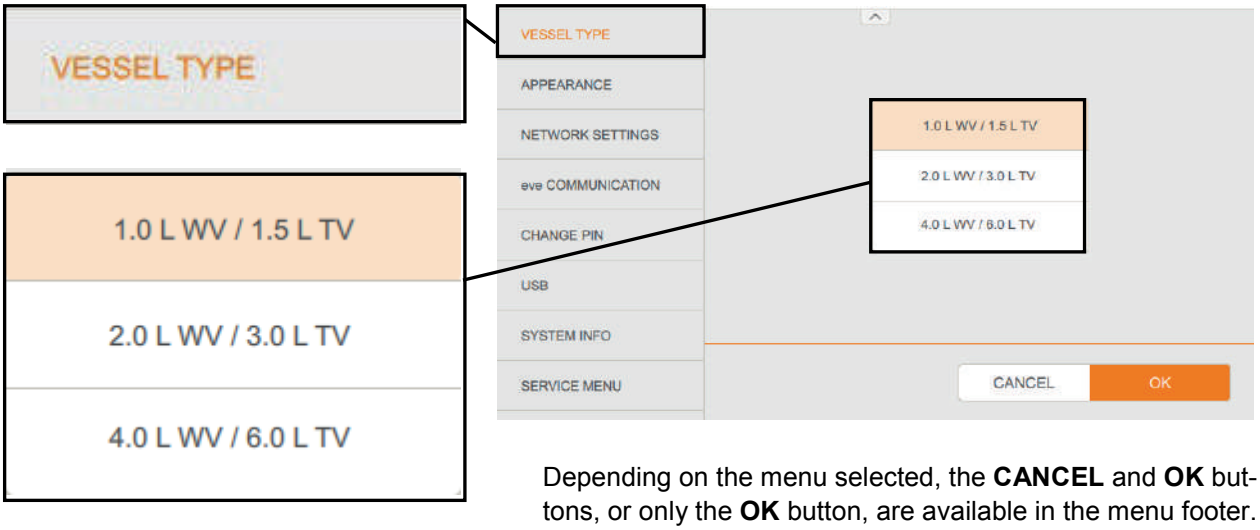
Alarm display

If alarms occur (equipment alarm or parameter alarm), they are signalled by a red exclamation mark highlighted in white on a red background. Pressing the symbol or swiping upwards opens the alarm menu.
For details, see the “Alarms – Equipment Alarm Menu” and “Parameter Alarms” sections.

10.1.1 Main Screen

Depending on the menu selected on the left side of the screen, the main screen displays the following:

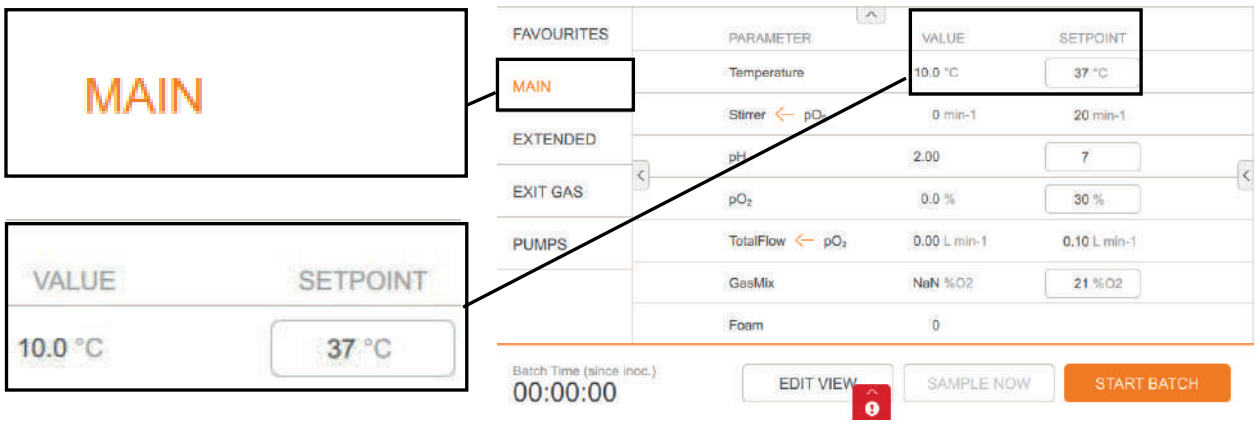
a) **Menus for system settings**, such as the *VESSEL TYPE* menu for setting the vessel size.



Depending on the menu selected, the **CANCEL** and **OK** buttons, or only the **OK** button, are available in the menu footer.

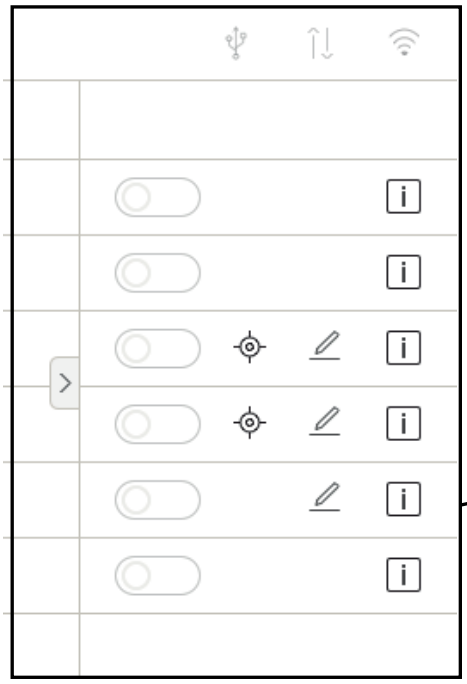
- **OK** saves the changes and closes the menu.
- **CANCEL** closes the menu without making any changes.

b) **Parameter group** with parameter actual values and setpoints, such as *MAIN* parameter group with actual values in the *VALUE* column and input fields for setpoints in the *SETPOINT* column

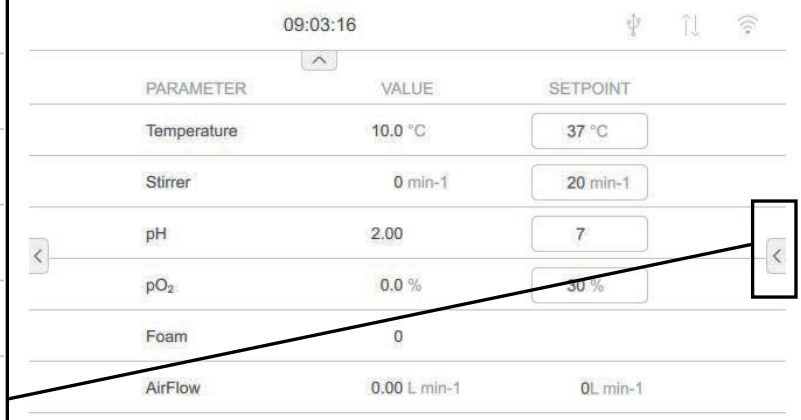


All menus and parameter groups can be selected by pressing them. The selected menu or parameter group is highlighted with a change of colour in the menu/group text from grey to orange.

Example to the left: *MAIN* parameter group



The arrow buttons at the edge of the main screen can be used to show or hide parts of the menu and display.



The figure to the left shows the example of the menu with parameter options, which becomes visible after pressing the arrow button on the right-hand edge of the screen (figure above).

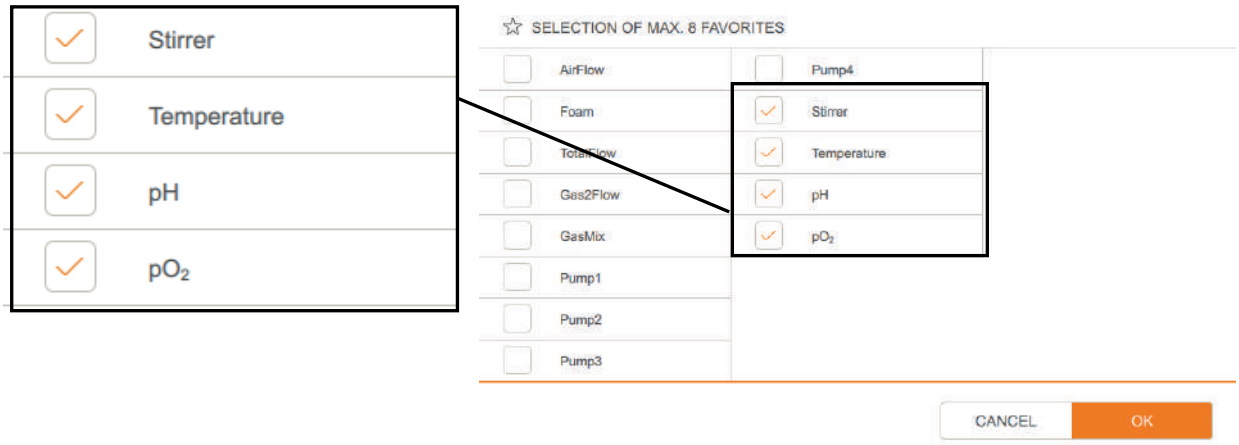
INFORMATION

Instead of using the arrow buttons, swiping movements to the left, right, upwards or downwards on the screen can also change the display.

10.1.2 EDIT VIEW

EDIT VIEW opens a menu with all available parameters.

Here, up to 8 parameters can be selected to appear in the *FAVOURITES* parameter group by checking the check box.



- **OK** confirms the selection and closes the menu.
- **CANCEL** closes the menu without making any changes.

10.1.3 START BATCH / INOCULATE / STOP BATCH

Pressing the **START BATCH** button starts the preparation phase for the Batch (bioprocess). The controller is activated. The current parameter settings are simultaneously logged in a log file, and recording of the actual values begins.

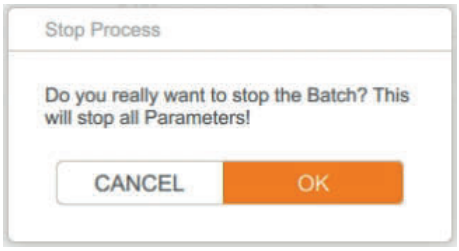
INFORMATION

Log files can be exported on a USB stick.

The button now changes function to **INOCULATE**. In this process phase, the parameters can be activated manually and individually.

When all preparations are finished, the bioreactor can be inoculated with the microorganisms. This is signalled by pressing **INOCULATE**. This means that this time corresponds to t = 0 of the Batch time**.**

The button now changes function to **STOP BATCH**.



After pressing **STOP BATCH**, the *Stop Process* dialogue appears asking for confirmation that the Batch is to be stopped, as well as the notice that doing so deactivates all parameters.

- **CANCEL** cancels the stop procedure without making any changes.
- **OK** finishes the batch, all parameters are deactivated and the controller is deactivated. Recording of actual values is ended, the button changes function back to **START BATCH**.

INFORMATION

Batch time remains visible until a new Batch is started or the equipment is switched off using the main switch.

10.1.4 SAMPLE NOW

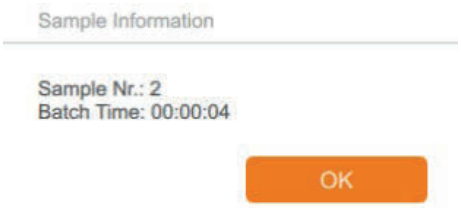
If a sample is removed from the culture vessel by hand, this can be signalled to the bioreactor by pressing **SAMPLE NOW.** This logs the sampling, and is visible in the log files for the batch. For details, see the “USB Data Export and Import from a USB Stick” section.

If the bioreactor is connected with the bioprocess platform software eve®, an *offline sample* is automatically created there.

For details on the procedure for process-suitable sampling, see the main chapter “Cultivation”, chapter “Sampling”.

The **SAMPLE NOW** button only becomes functional after pressing **START BATCH**. This means that it can only be used during a batch.

SAMPLE NOW generates consecutive numbers for all samples and logs them with the batch time since inoculation as the time stamp. If sampling takes place before inoculation, the current time is logged instead of the batch time since inoculation.

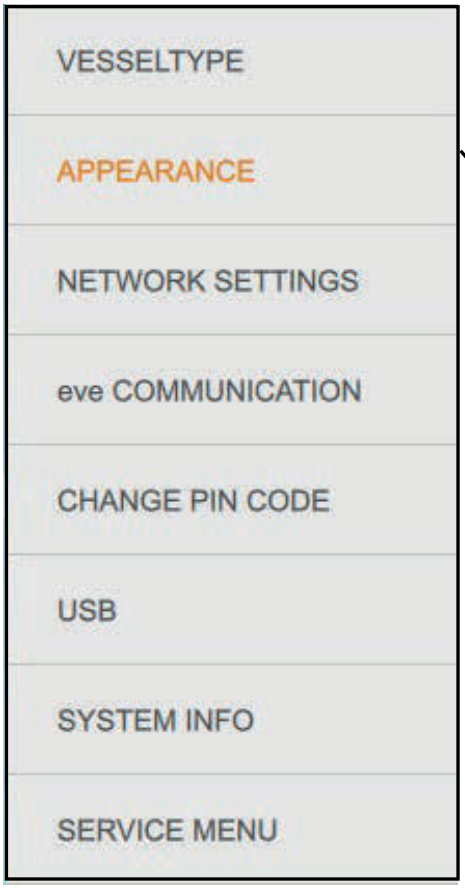


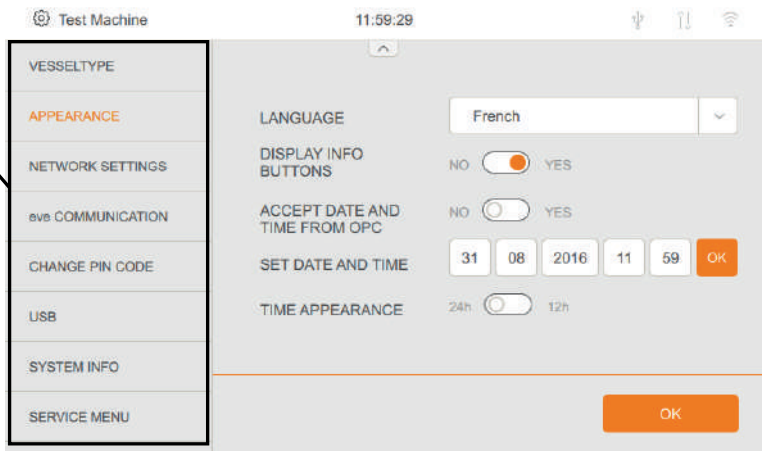
This means that the *Sample information* dialogue appears and displays how long the batch has been running since inoculation (*Batch Time….)*, and how many samples have been taken (*Sample Nr.…..*), or how many times **SAMPLE NOW** has been pressed since **START BATCH** was pressed.

OK closes the dialogue.

10.2 Menus for System Settings

There are eight menus for system settings, of which six are available for the operator.





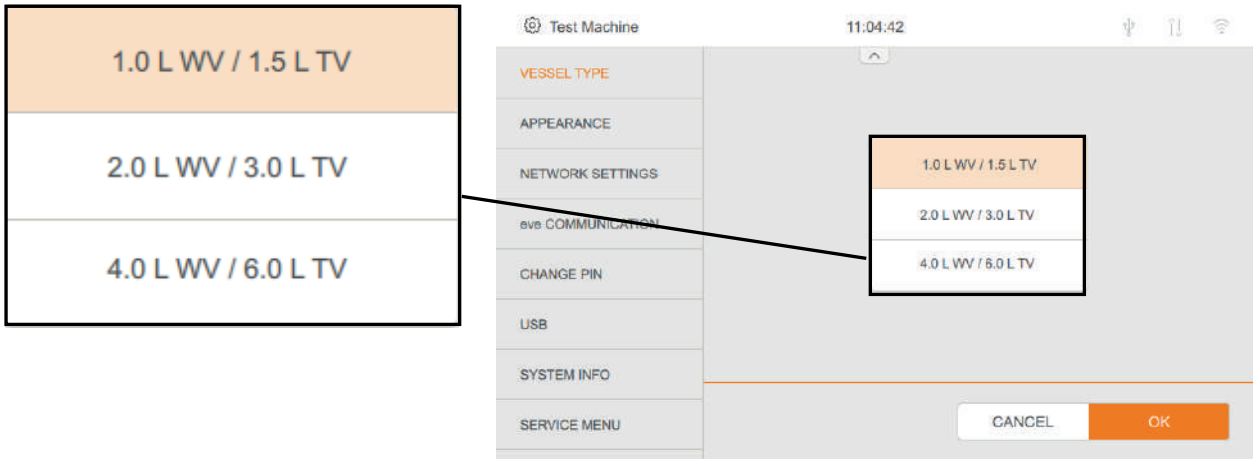
- **VESSEL TYPE**: Selection of the culture vessel used
- **APPEARANCE**: Display settings, including language and time
- **NETWORK SETTINGS**: Network configuration
- **eve COMMUNICATION**: Configuration of the OPC UA server for communication with the bioprocess platform software eve® by the equipment manufacturer.
- **CHANGE PIN CODE**: Password settings
- **USB**: Export files on a USB stick or load updates and additional packages from a USB stick.
- **SYSTEM INFO**: Information about system versions and uptime
- **SERVICE MENU**: Functions for authorised service partners of the equipment manufacturer, only accessible with the relevant password

INFORMATION

Depending on the menu selected, the **CANCEL** (leave the menu without changes) and **OK** (save changes and leave the menu) buttons, or only the **OK** button, are available in the menu footer.

10.2.1 VESSEL TYPE – Selecting a Culture Vessel

The culture vessel used is set in the *VESSEL TYPE* menu. There are three culture vessel sizes.

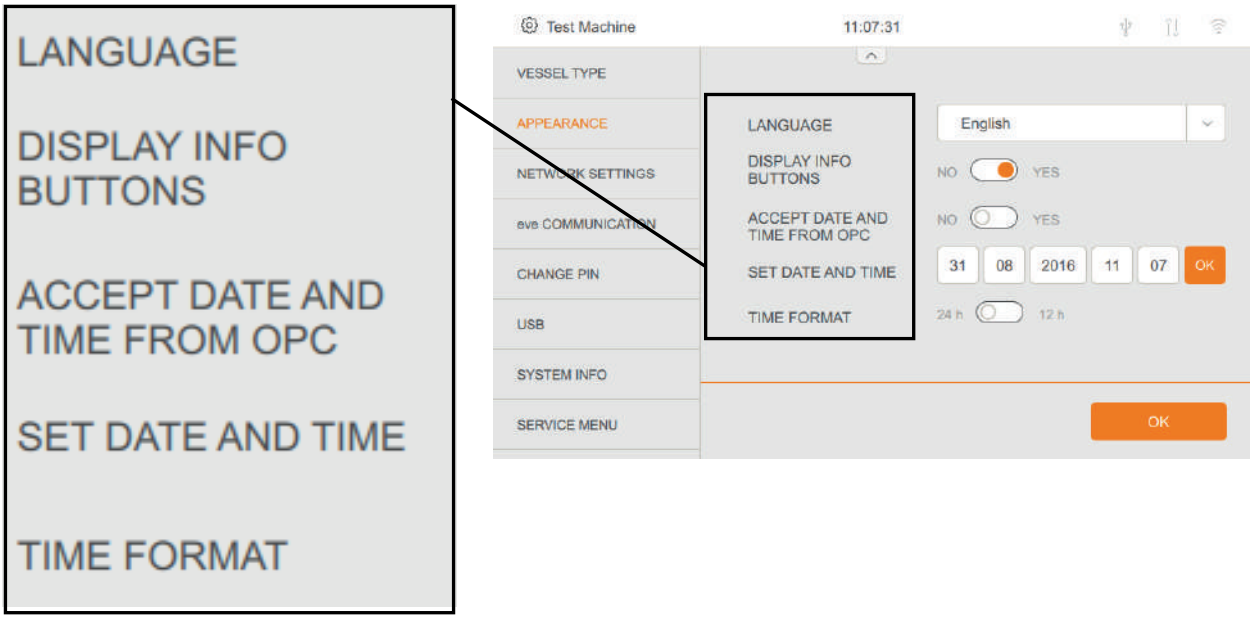


ATTENTION

With the selection of the culture vessel used, the permitted limit values and control settings for the corresponding vessel size are configured in the background. If the vessel size is set incorrectly, it could cause undesired behaviour from the control.

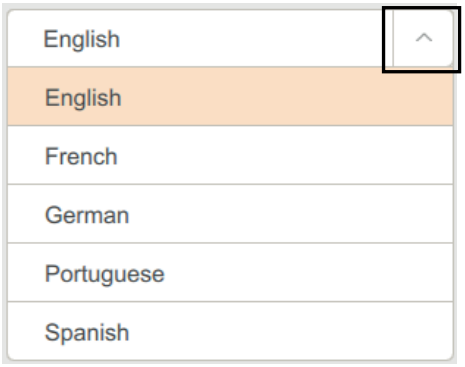
10.2.2 APPEARANCE – Display Settings

A variety of display settings can be made in the *APPEARANCE* menu.



LANGUAGE

Selection of the display language



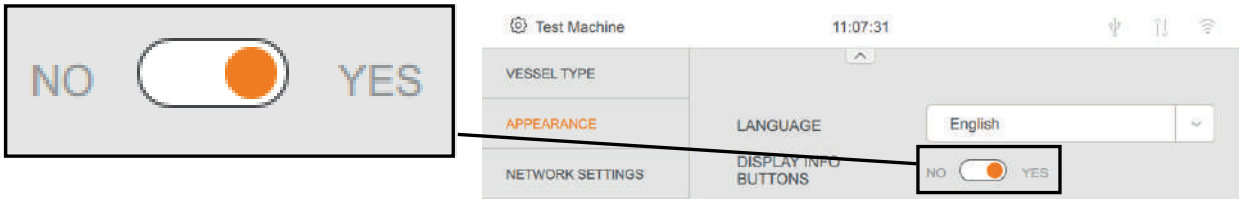
The desired display language can be selected using the pull-down menu. The languages in the pull-down menu are always displayed in English.

INFORMATION

Additional languages can be downloaded from the INFORS HT website to a USB stick and loaded to the equipment from the **USB** menu. If the desired language is not available, contact the local service partner of the equipment manufacturer.

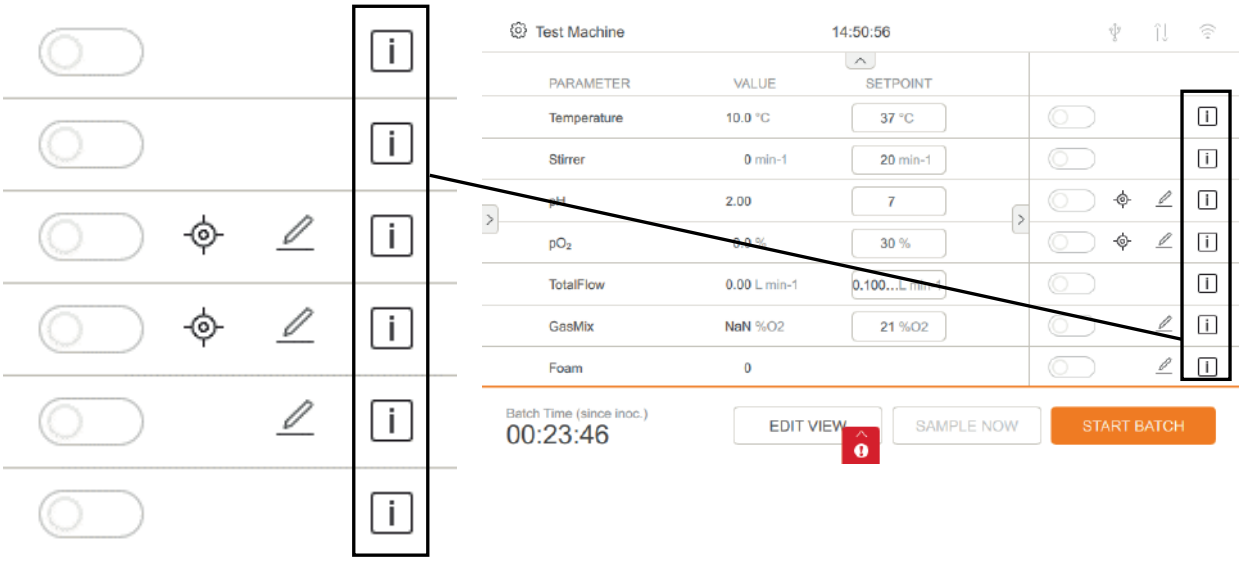
DISPLAY INFO BUTTONS

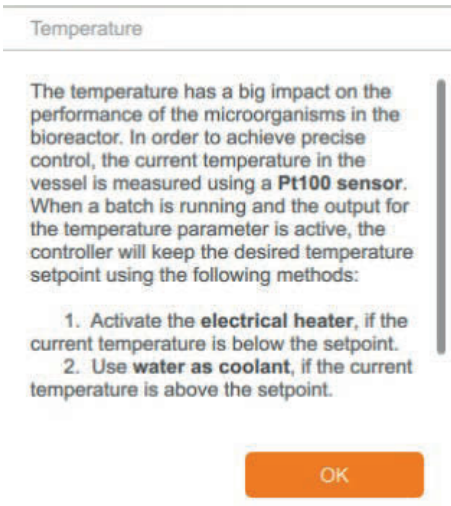
Switching the on-screen help off or on



This display of the info buttons for the on-screen help for the various parameters is switched on (YES) or off (NO) using the switch.

If the display is switched on, the info buttons also appear in the main screen.





After pressing an info button, a dialogue appears containing basic information on the selected parameter, example to the left: *Temperature* parameter.

ACCEPT DATE AND TIME FROM OPC

Switching on or off the acceptance of date and time from a PC

If this function is switched on, a connected OPC UA client such as the bioprocess platform software eve® can overwrite the date and the time of the bioreactor for synchronisation purposes.

INFORMATION

In this case, the date and the time of the bioreactor cannot be set manually (SET DATE AND TIME).

SET DATE AND TIME

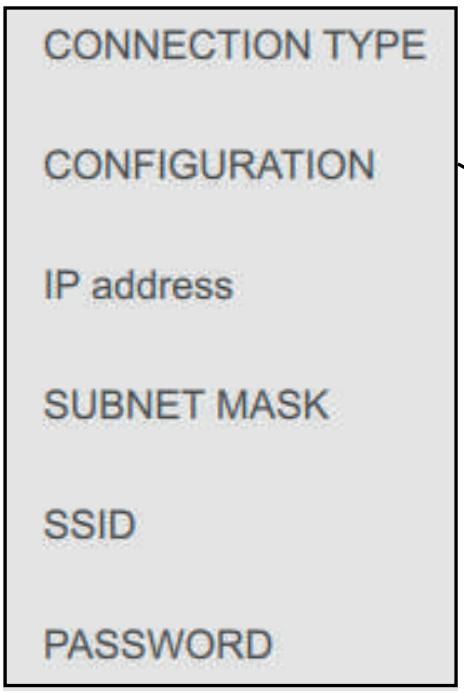
Manually enter the date and time. Can only be done provided *ACCEPT DATE AND TIME FROM OPC* is not switched on.

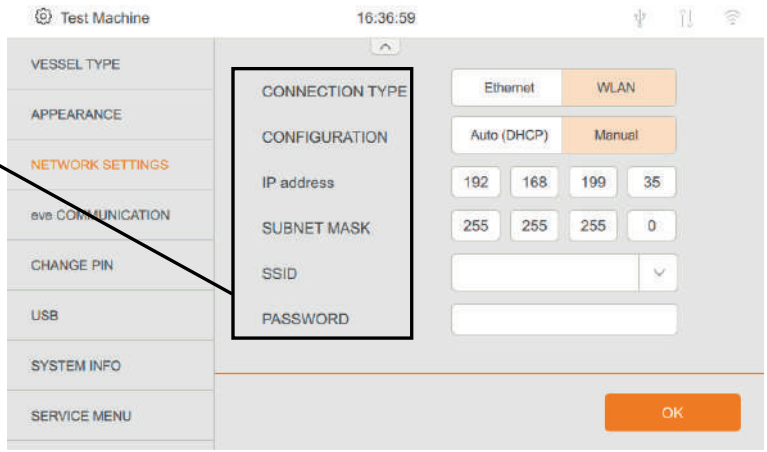
TIME FORMAT

Switch between 12 h and 24 h time formats

10.2.3 NETWORK SETTINGS

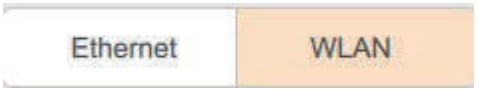
The network connection of the bioreactor is configured in the *NETWORK SETTINGS* menu.





INFORMATION

If the bioreactor is to be integrated into an existing network, the network specifications are to be followed and the corresponding settings are to be used. Please consult your network administrator.



CONNECTION TYPE

Select whether a cable connection (**Ethernet**) or cable-free (**wireless**) network connection is to be used.
An optional wireless adaptor is required for a connection with a wireless network.

CONFIGURATION

Determine whether the network connection is to be automatically configured (**Auto (DHCP)**) or needs to be set up manually (**Manual**).
A DHCP server is required in the network for automatic configuration with the DHCP protocol. Please consult your network administrator.

IP ADDRESS

Displays the allocated IP address during automatic configuration (*Auto (DHCP)*), or can be used to enter the IP address for manual configuration (*Manual*).

SUBNET MASK

Displays the subnet mask address during automatic configuration (*Auto (DHCP)*), or can be used to enter the subnet mask for manual configuration (*Manual*).

SSID

Selection of the wireless network when using a wireless adaptor.

PASSWORD

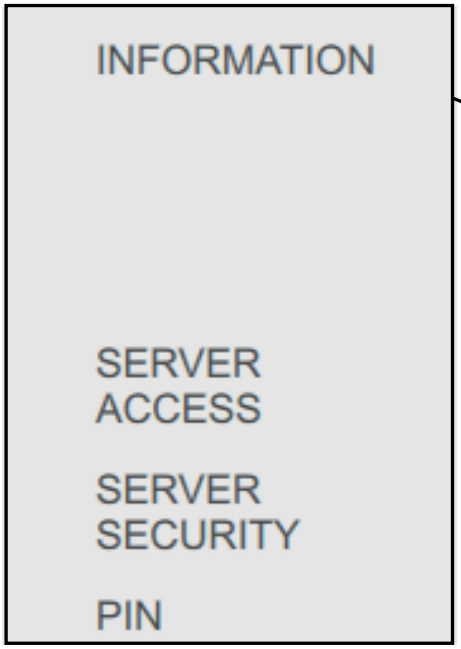
Password/key for connection with the wireless network when using a wireless adaptor.

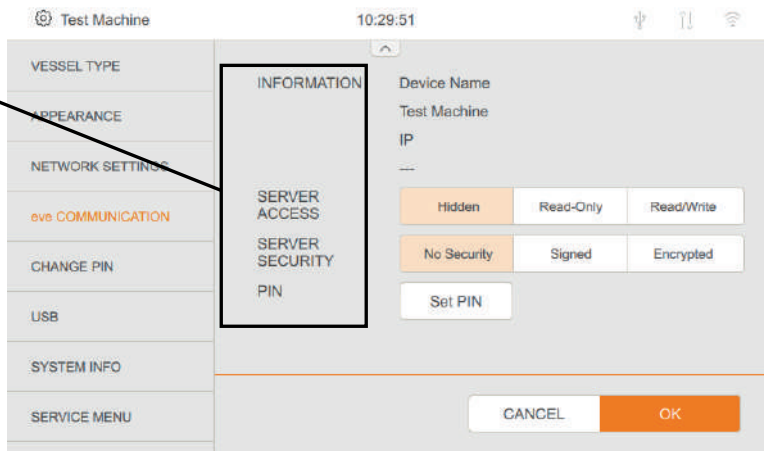
INFORMATION

The network connection can be used to connect the equipment with the bioprocess platform software eve®.

10.2.4 eve COMMUNICATION – Communication Settings

In the eve COMMUNICATION menu, permissions for server access as well as their security settings for communication with the bioprocess platform software eve® are set.





INFORMATION

Display of the equipment name (*Device Name*) and its network address (*IP*, configuration under *NETWORK SETTINGS*). This information is required for configuration of the connection in eve® (equipment manufacturer's bioprocess platform software)

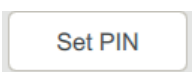
SERVER ACCESS

Determine whether the bioreactor is invisible (**Hidden**), only available for read access (**Read Only**) or available for read and write access (**Read/Write**) in the OPC UA.

SERVER SECURITY

Determine whether communication between the bioreactor and the OPC UA client (e.g. eve®) is unencrypted (**No Security**), signed (**Signed**) or encrypted (**Encrypted**).

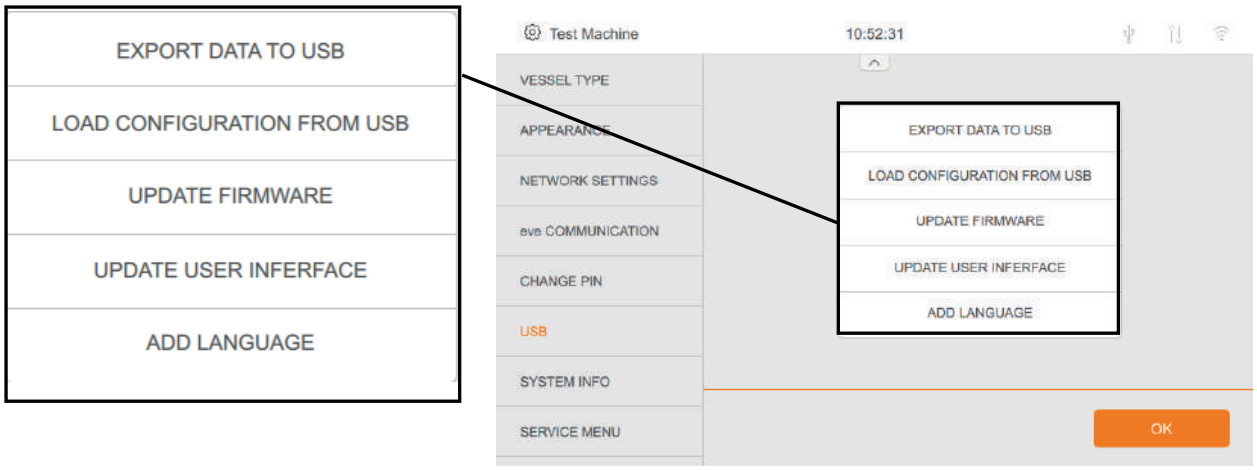
SET PIN



For optional safety of communication with eve®

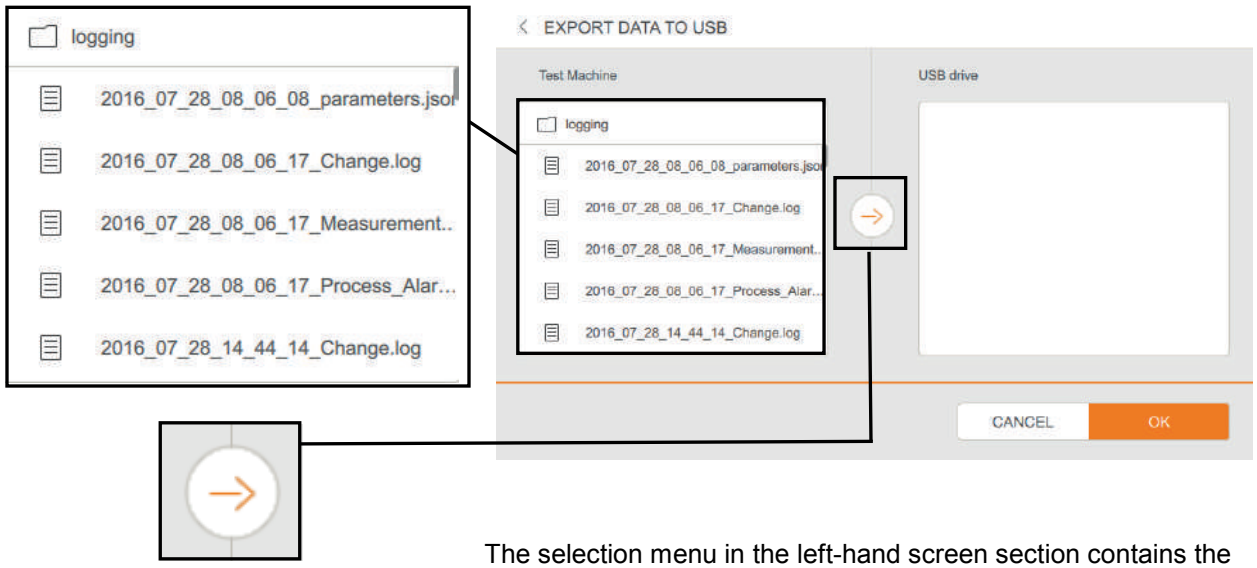
10.2.5 USB Data Export and Import from a USB Stick

In the *USB* menu, a USB stick connected with the equipment's USB port can be used to import or export data.



EXPORT DATA TO USB

Opens the menu for data export.



The selection menu in the left-hand screen section contains the files that can be moved across to the USB stick using the arrow button in the middle.

Four files are created per batch and are ready for export. Each file name contains the start date and start time of the batch:

yyyy	=	Year
mm	=	Month
dd	=	Day
hh	=	Hours
ii	=	Minutes
ss	=	Seconds

The four files contain the following:

1) **yyyy_mm_dd_hh_ii_ss_Parameters.json**

Settings of the bioreactor at batch start in JSON format. This file can be archived or loaded again later using *LOAD CONFIGURATION FROM USB*.

2) **yyyy_mm_dd_hh_ii_ss_Change.csv**

Log of the changes during the batch, e.g. manual input of setpoints, in CSV format. In combination with the initial state at batch start (parameters.json) the current configuration can be determined at any time.

The columns of the CSV file are:

- DateTime: Absolute date and time
- Parameter: Parameter that was changed
- Property: Property of the parameter that was changed
- NewValue: Property of the newly allocated value
- OldValue: The old value of the property

3) **yyyy_mm_dd_hh_ii_ss_Measurement.csv**

Log of the actual values of all parameters during the batch in CSV format. The recording interval is 1 min. If higher precision is required, SCADA software, e.g. the bioprocess platform software eve®, can be connected via OPC UA and used for recording.

The columns of the CSV file are:

- DateTime: Absolute date and time
- ProcessTime: Relative time to batch start (*batch time*)

– <ParameterName>: Actual value of the corresponding parameter

4) **yyyy_mm_dd_hh_ii_ss_Process_Alarm.csv**

Log of all alarms that occurred during the batch (e.g. deviations from the setpoints and actual values) and events (e.g. sampling) in CSV format.

The columns of the CSV file are:

– DateTime: Absolute date and time
– AlarmType: Type of alarm or event
– ProcessTime: Relative time to batch start (*batch time*)
– EndAlarmTime: Time at which the alarm state was resolved
– ConfirmedTime: Time at which the alarm was confirmed on the operating panel

LOAD CONFIGURATION FROM USB

Opens the menu for data import from a USB stick for the data import of the parameter configuration. This also contains the cascades and the PID settings.

UPDATE FIRMWARE

Carries out an update of the controller firmware. Observe the instructions on the operating panel.

ATTENTION

Only carry out an update of the controller firmware after consultation with an authorised INFORS HT service partner. Incorrect application could damage the equipment.

UPDATE USER INTERFACE

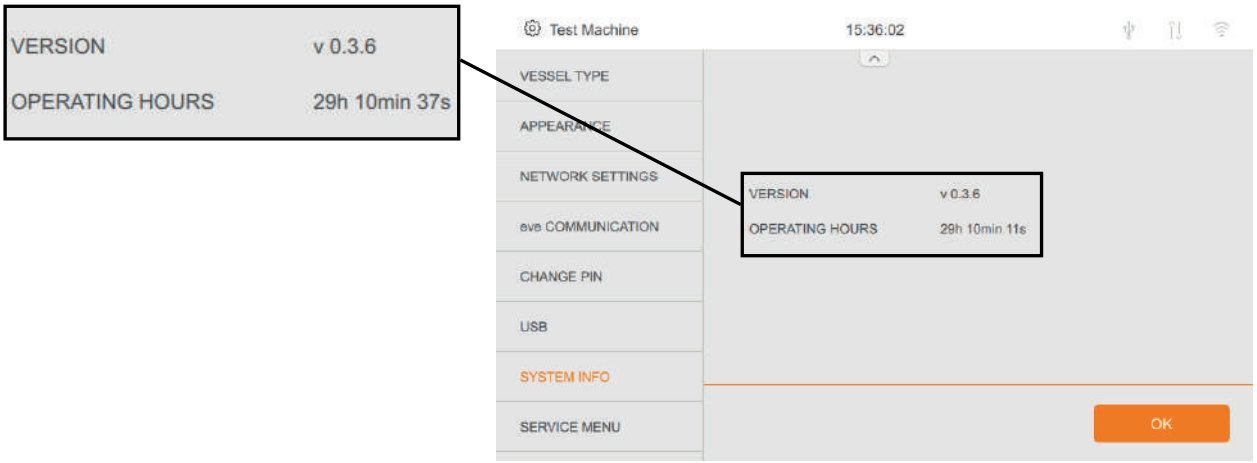
A selection menu for data import appears. An update for the operating panel can be selected, e.g. downloaded from the INFORS HT download centre or provided by an authorised INFORS HT service partner.

ADD LANGUAGE

A selection menu for data import appears. A language can be added or updated, e.g. downloaded from the INFORS HT download centre or provided by an authorised INFORS HT service partner.

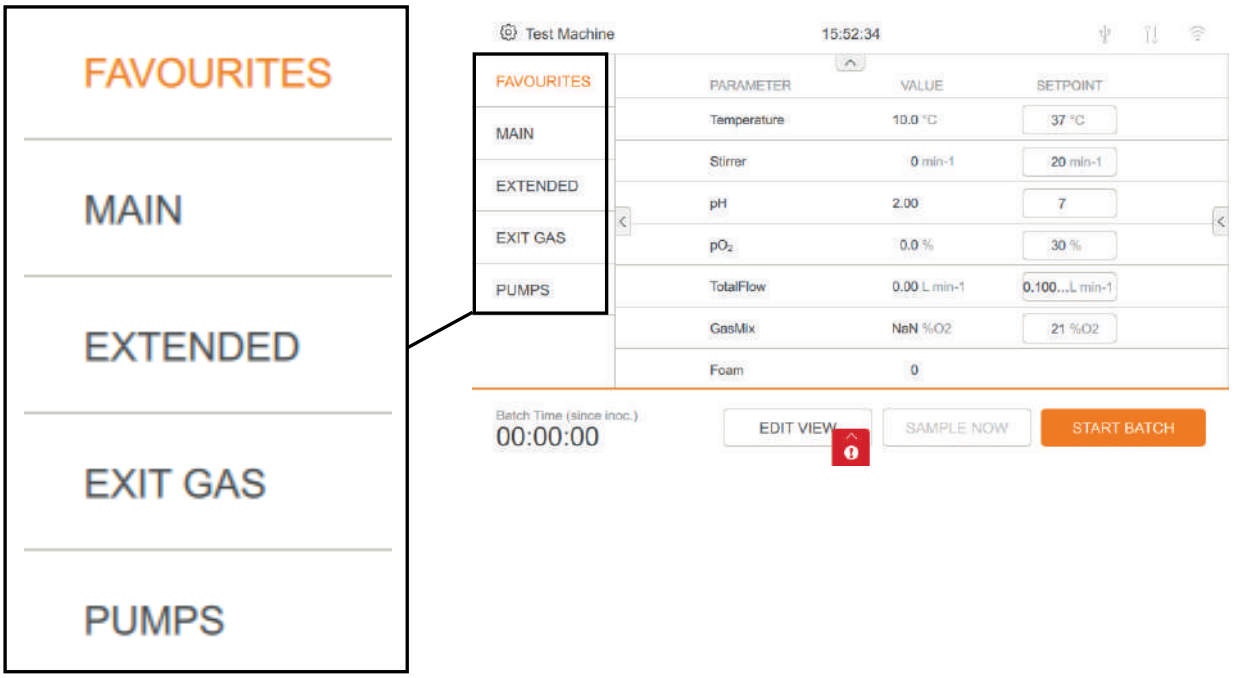
10.2.6 SYSTEM INFO – System Information

In the *SYSTEM INFO* menu, the currently installed version of the firmware and the operating hours since start-up are displayed.



10.3 Parameter - Parameter Groups

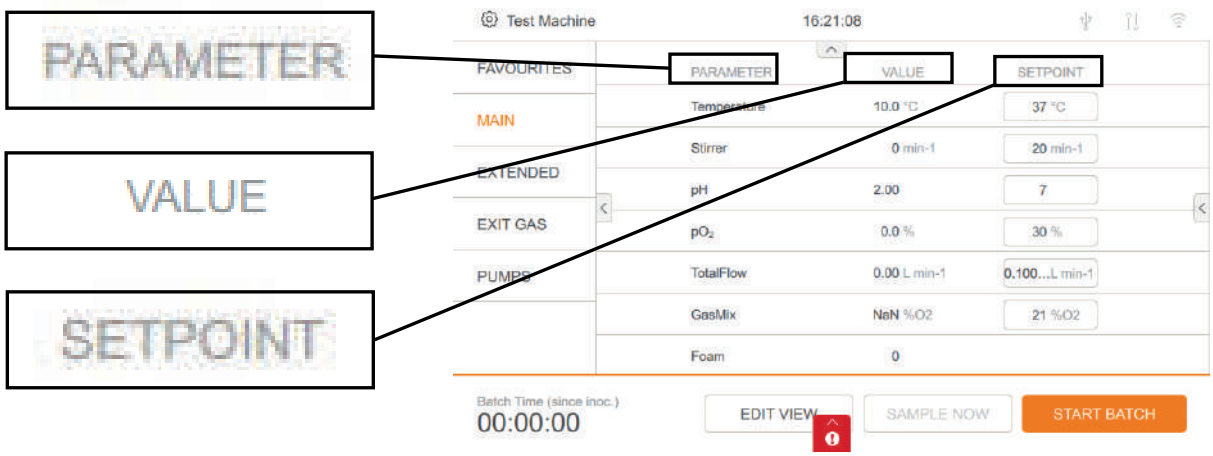
On the main screen, up to eight parameters can be simultaneously monitored and controlled. The parameters are divided into five parameter groups:



- **FAVOURITES**: Allows you to compile up to eight parameters from the other four parameter groups. This can be done using the **EDIT VIEW** button. For details, see the “EDIT VIEW” section.
- **MAIN**: Contains the *Temperature, Stirrer, pH, pO_2, TotalFlow, Gasmix* and *Foam* parameters.
- **EXTENDED**: Contains the *AirFlow*, *Gas2Flow* parameters, as well as the optional parameters *Balance* and *Turbidity*, if available.
- **EXIT GAS**: Contains the *Exit GasO_2* and *Exit GasCO_2* parameters, provided the exit gas analysis option is available.
- **PUMPS**: Contains the *Pump1* to *Pump4* parameters and also offers the *FILL* and *EMPTY* functions

10.3.1 Parameters – Displays and Functions

Irrespective of the selected parameter group, every parameter menu has the same three columns.



- **PARAMETER**: Display of the parameter name
- **VALUE**: Display of the actual value of the parameter
- **SETPOINT**: Entry of the max. setpoint of the parameter

If the right-hand display is shown, further functions are available depending on the selected parameter group and parameter.

Operation

ON/OFF

switches control of the selected parameter on or off

> **INFORMATION**
>
> ON/OFF is only available when a Batch is running. First start the batch with START BATCH and, if necessary, INOCULATE.

Calibrating

opens the calibration menu of the selected parameter.

> **INFORMATION**
>
> The calibration function is only available for the pH, pO_2 and Turbidity parameters.

Operation is described in the “Calibration” section.

Editing

opens the editor menu with the various settings for the selected parameter. Not all parameters have an editor menu.
Amongst other options, cascades can be set, PID settings can be adjusted, parameter alarms can be switched on or off or the pump functions can be selected.

The settings are described along with the corresponding parameter in the later parameter sections.

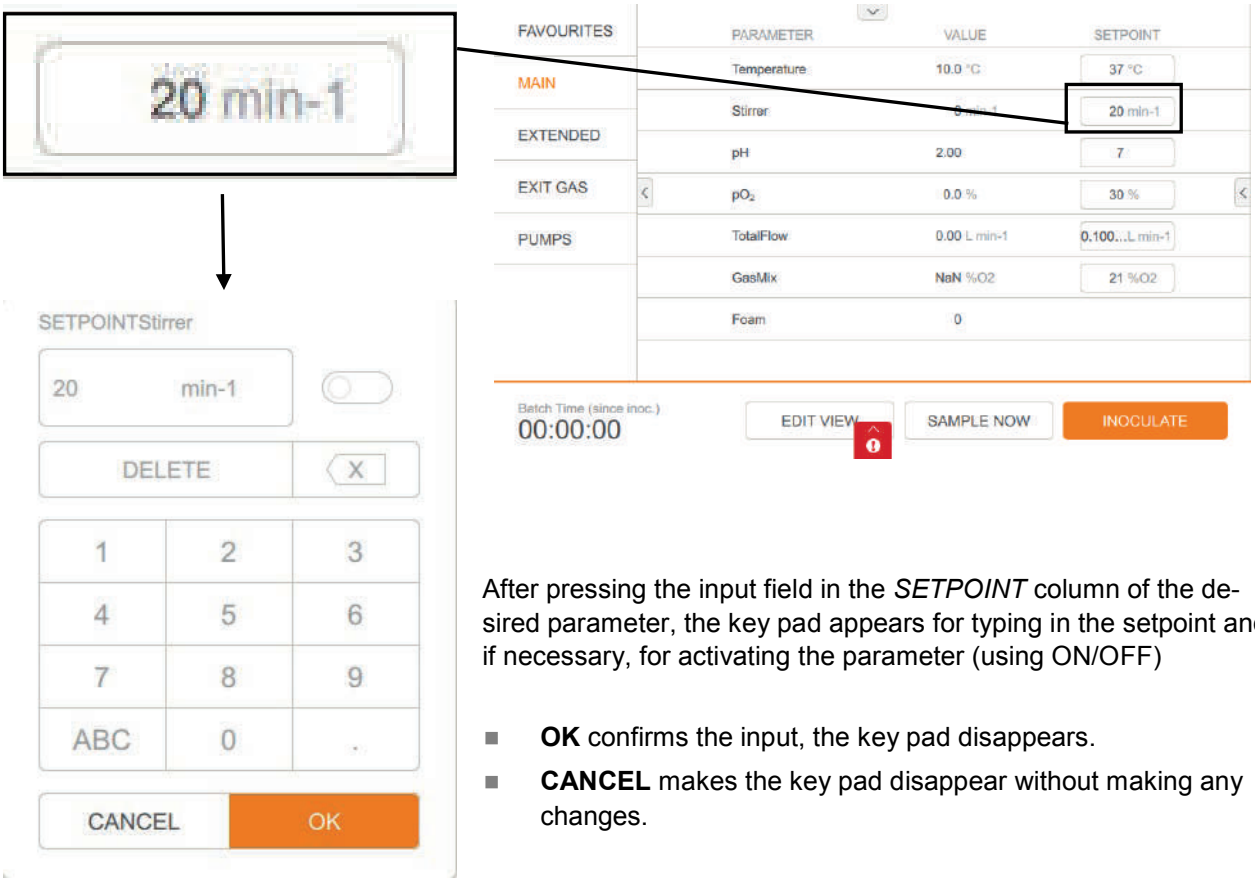
Information

opens a dialogue containing basic information on the selected parameter.

> **INFORMATION**
>
> Display of the info button is switched on or off in the *APPEARANCE* menu for system settings.

10.3.2 SETPOINT - Setting the Setpoint

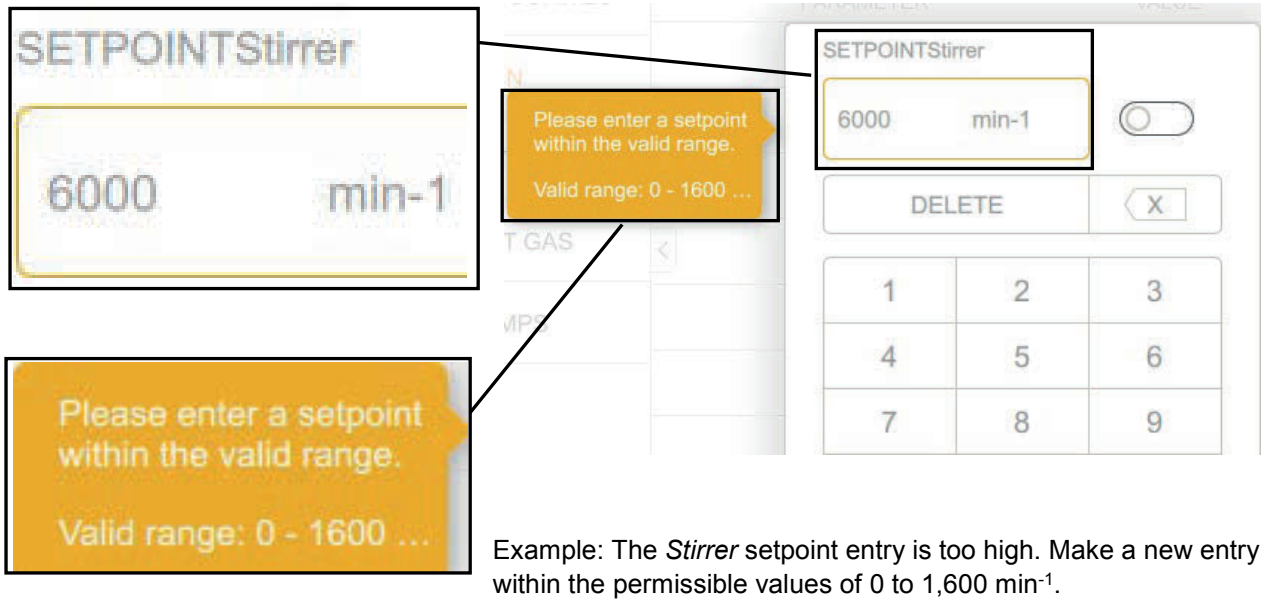
The setpoints can be entered in any operating state of the equipment for parameters that are not controlled via cascade and have a controller output. Parameter control is however only active when a batch has been started using **START BATCH** and the corresponding parameter has been activated using **ON/OFF**.



After pressing the input field in the *SETPOINT* column of the desired parameter, the key pad appears for typing in the setpoint and, if necessary, for activating the parameter (using ON/OFF)

- **OK** confirms the input, the key pad disappears.
- **CANCEL** makes the key pad disappear without making any changes.

If an inadmissible setpoint is entered, an error message is generated that prompts a correct entry within the permissible parameter setpoints.



Example: The *Stirrer* setpoint entry is too high. Make a new entry within the permissible values of 0 to 1,600 min^{-1}.

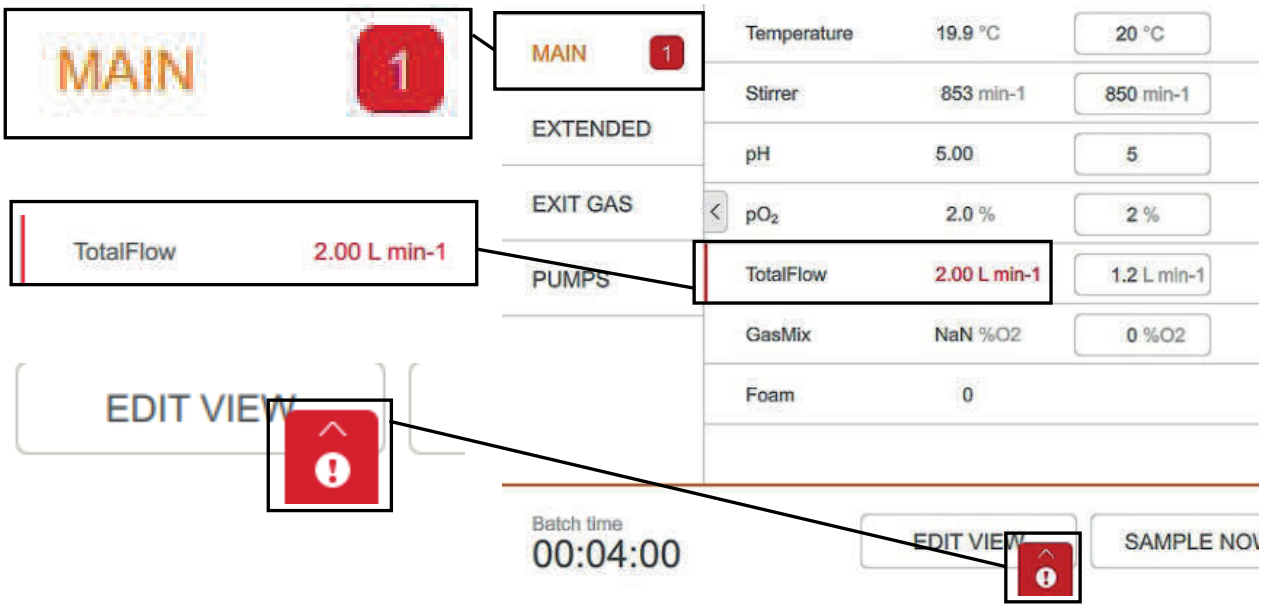
10.3.3 Parameter Alarms

If a parameter is activated and the Batch inoculated, parameter alarms are generated after a predefined waiting time if there are unexpected deviations from the actual values and setpoints.

Alarm generation can be switched on or off in the editor menu of the corresponding parameter.

Example to the left: Editor menu for pH parameter, alarm generation switched on.

Parameter alarms are displayed as follows:



1) In the parameter group that contains the parameter in question, a number on a red background appears. This indicates the number of existing parameter alarms.
2) The parameter in question is displayed with a red bar and a red actual value.
3) A red exclamation mark highlighted in white on a red background appears in the footer.

Pressing the symbol or swiping upwards opens the *Equipment Alarm* menu. For details, see the “Alarms – Equipment Alarm Menu” section.

Parameter alarms are also logged in the batch log file, see the “USB data export and import from a USB stick” section.

Parameter alarm limit factory settings

Parameters	Alarm limits
Temperature	2 °C
Stirrer	50 min^{-1}
pH	0.5
pO2	10 %
TotalFlow	0.3 L min^{-1}
GasMix	10 %
AirFlow	0.3 L min^{-1}
Gas2Flow	0.3 L min^{-1}

10.3.4 Cascades

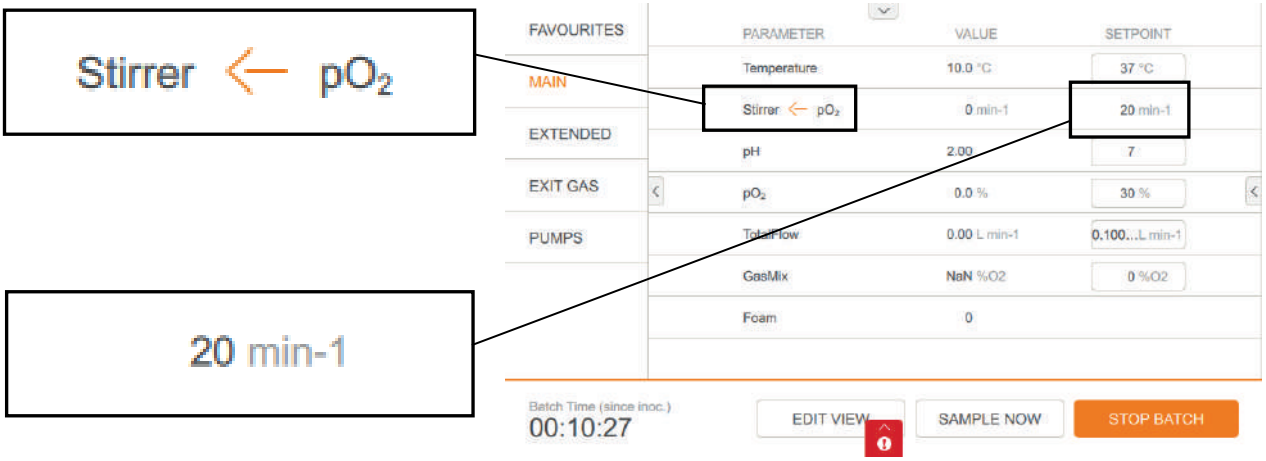
Cascades can be configured for some parameters. A cascade can be used to assign a parameter to another parameter as an actuator.

Example:

For control of the pO_2 by changing the *Gasmix* parameter, a cascade to the *Gasmix* parameter is configured for the pO_2. If the pO_2 actual value is below the prescribed setpoint, the *Gasmix* is increased by the controller until the desired setpoint is reached for the pO_2.

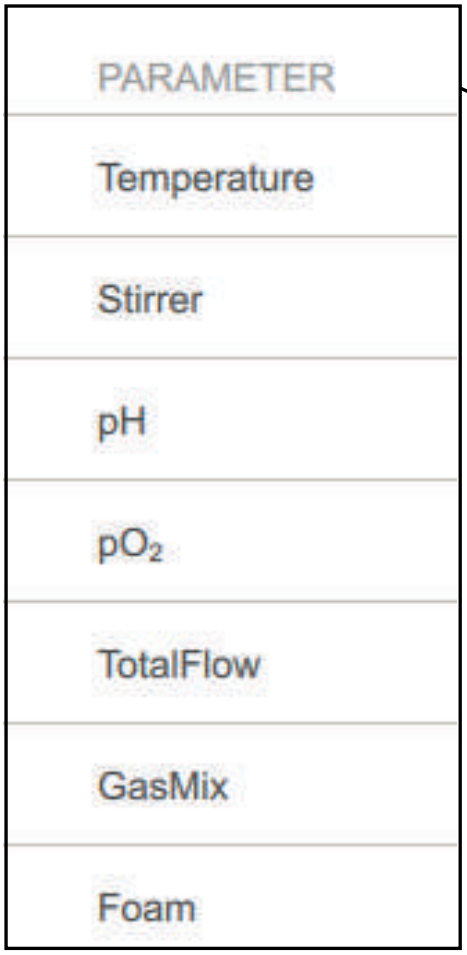
The cascades can be configured using the editor menu of the parameter. The procedure is described in the parameter description for the parameters for which this is possible.

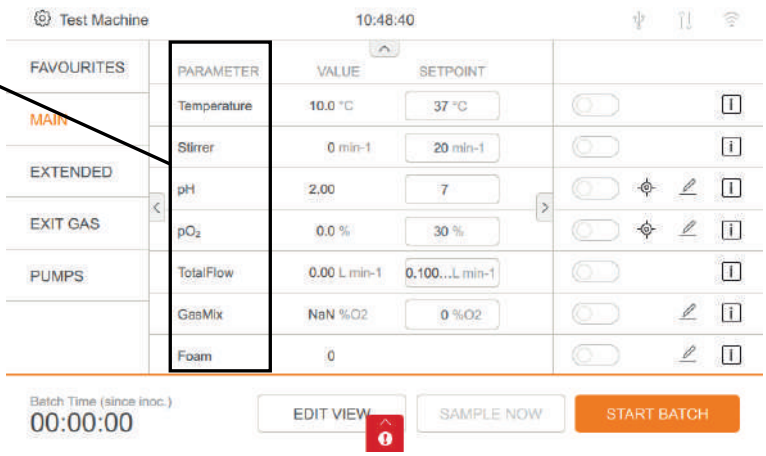
Parameters that are used in a cascade are identified in the main menu with an arrow and the name of the controlling parameter, and manual setpoint entry is deactivated.



Example: *Stirrer* is used in a cascade for pO2 control. It is not possible to enter a setpoint for *Stirrer*.

10.4 MAIN Parameter Group





The *MAIN* parameter group contains all parameters that are available as standard, as well as the *GasMix* and *TotalFlow* parameters, which are only functional when using a second (optional) gas.

10.4.1 Temperature

Measures and controls the temperature in the culture vessel.

10.4.2 Stirrer

Measures and controls the speed of the stirrer. Rotation speed depends on factors such as the size of the motor, vessel volume, culture viscosity, number and kind of impellers etc.

Stirring speed is often used in a cascade for pO_2 control. Cascades for pO_2 control can be configured in the editor menu of the pO_2 parameters.

10.4.3 pH

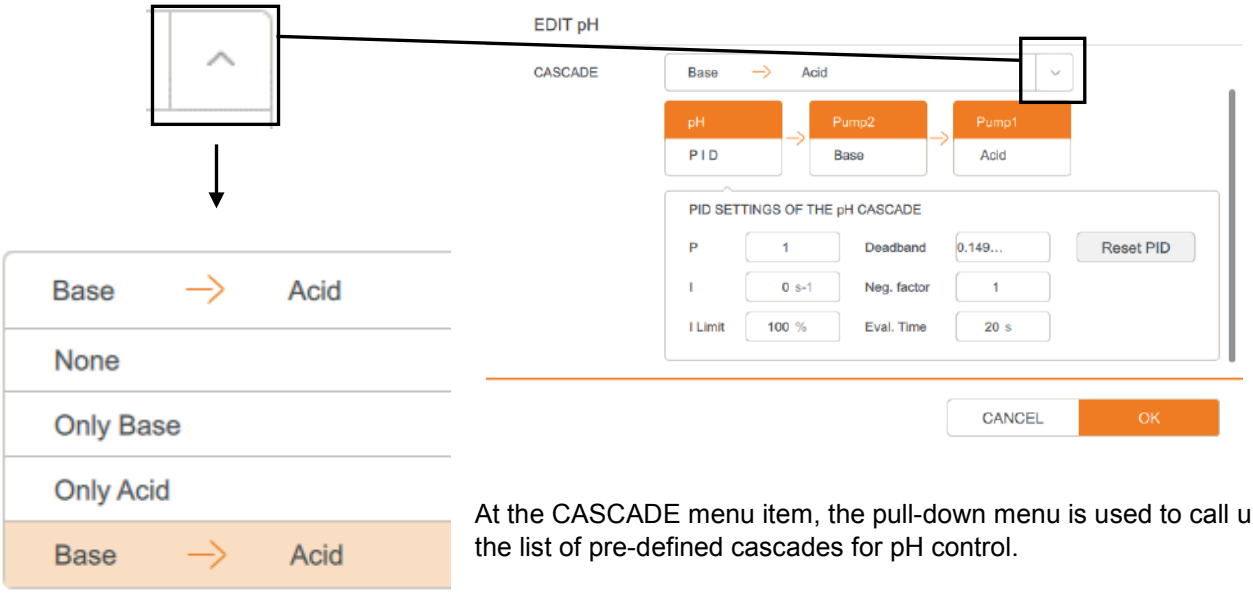
Measures and controls the pH in the culture vessel within a range of 2 to 12.

For details on the safety, technical data, usage and maintenance requirements for the pH sensors, see the separate documentation provided by the sensor manufacturer.

The pH control can be configured using a cascade and takes place by default by adding acid and base via the two peristaltic pumps *Pump1/Acid* and *Pump2/Base*. For details on the pumps, see the “PUMPS Parameter Group” section.

Settings

The settings for the cascade are carried out in the editor menu of the parameter.



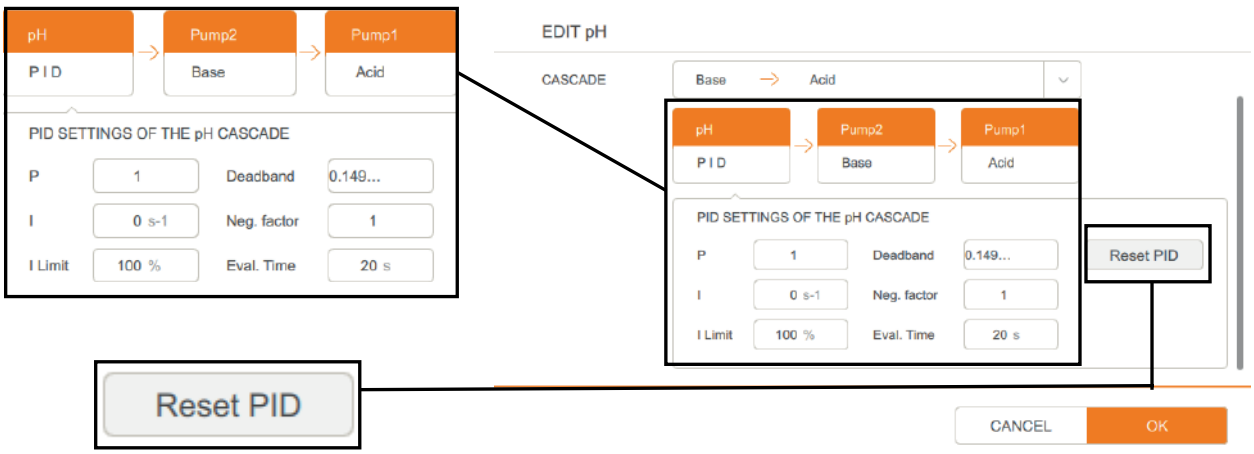
At the CASCADE menu item, the pull-down menu is used to call up the list of pre-defined cascades for pH control.

The following settings are available for selection:

- **None**: No control, pH is only measured.
- **Only Base**: pH control only takes place by adding base from *Pump2*.
- **Only Acid**: pH control only takes place by adding acid from *Pump1*.
- **Base – Acid**: The standard setting is that pH control takes place by first adding base and then adding acid.

The selected setting is represented visually. In the below example, the standard setting with control via the acid and base pumps is depicted.

The PID menu is activated.



The PID settings can be adjusted here as required or, if necessary, can be reset to factory settings using **RESET PID**.

For details on the PID controller, see the “PID Controller - Basics” section and associated sub-sections.

After setting the desired cascade, entries are confirmed using **OK**.

10.4.4 pO_2

Measures the dissolved oxygen in the culture. Unlike measurements such as pH, which are calibrated to absolute measurement values, the oxygen measurement is always calibrated to a relative reference point. For this purpose, the calibration is set to 100 % relative oxygen saturation, usually with air at max. stirring speed and maximum gas flow rate.

The actual concentration of dissolved oxygen in mmol L^{-1} may therefore vary at 100 % saturation, depending on the process.

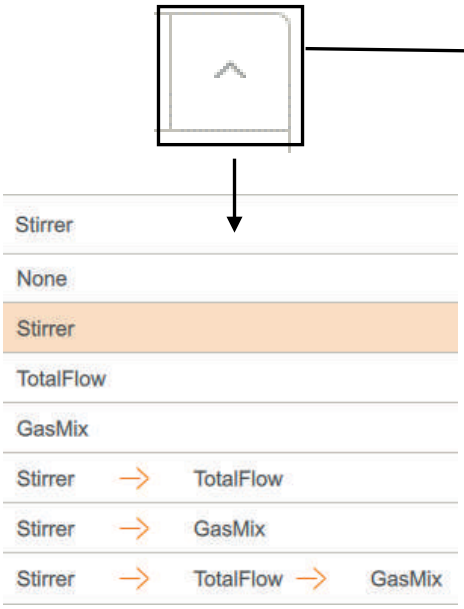
For details on the safety, technical data, usage and maintenance requirements for the pO_2 sensors, see the separate documentation provided by the sensor manufacturer.

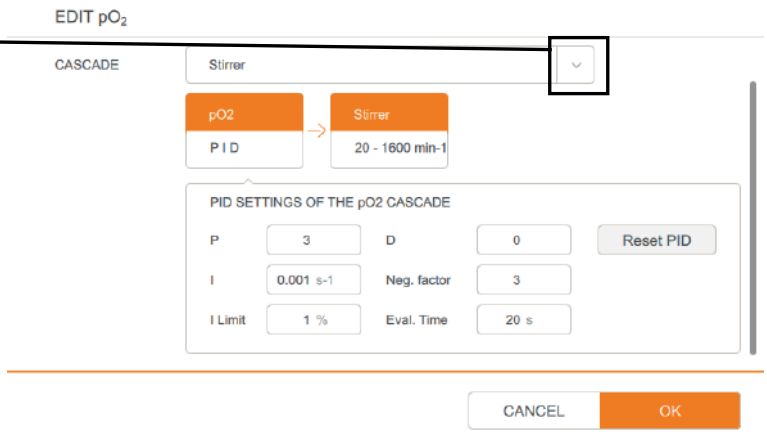
Since the pO_2 cannot be directly influenced by the bioreactor, the PID controller of the pO_2 parameter must be assigned an actuator. This takes place using cascades with other parameters, such as *Stirrer* (stirrer speed), *TotalFlow* (gas flow) or *GasMix* (gas mixture).

Settings

The settings for the cascade are carried out in the editor menu of the parameter.

Operation





At the CASCADE menu item, the pull-down menu is used to call up the list of pre-defined cascades for pO_2 control.

The following settings are available for selection:

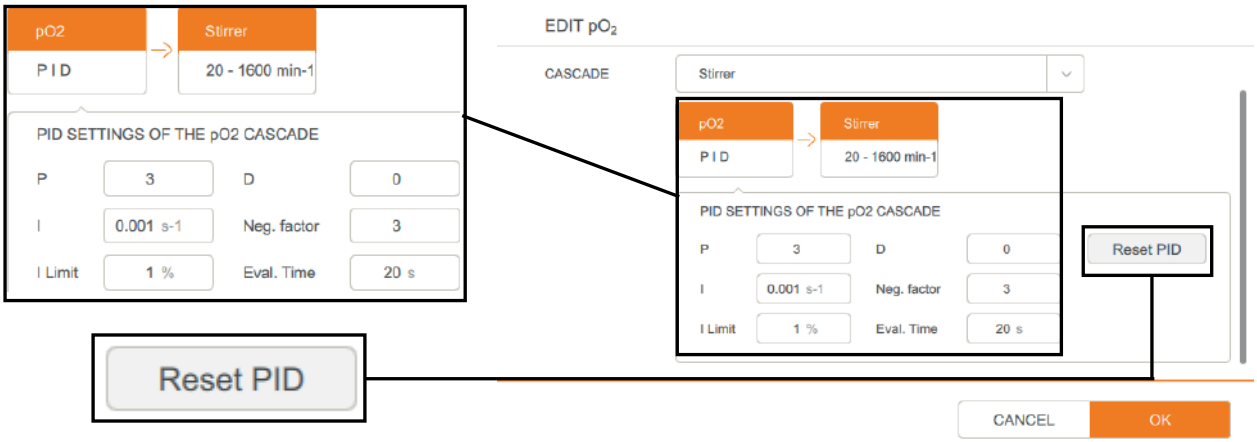
- **None**: No control, pO_2 is only measured.
- **Stirrer**: pO_2 is controlled using *Stirrer*
- **TotalFlow**: pO_2 is controlled using *TotalFlow*
- **GasMix**: pO_2 is controlled using *GasMix*.

Serial cascades

- **Stirrer – TotalFlow**: pO_2 is first controlled by *Stirrer* and, after reaching its maximum, it is controlled by *TotalFlow*.
- **Stirrer – Gasmix**: pO_2 is first controlled by *Stirrer* and, after reaching its maximum, it is controlled by *Gasmix*.
- **Stirrer – TotalFlow – GasMix**: pO_2 is first controlled by *Stirrer* and, after reaching its maximum, it is controlled by *TotalFlow* and, after reaching its maximum, it is controlled by *Gasmix*.

The selected setting is represented visually. In the below example, the setting with control using *Stirrer* (stirrer speed) is depicted.

The PID menu is activated.

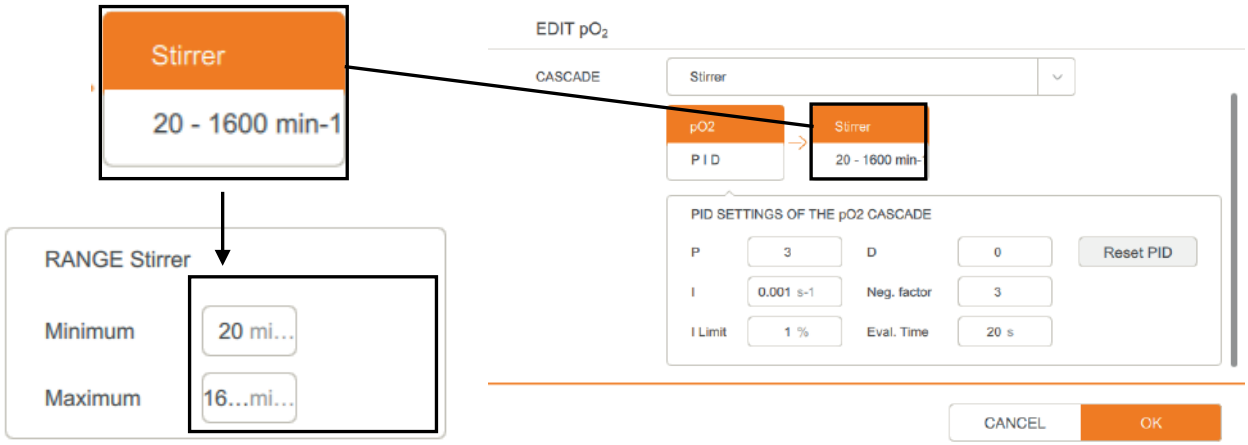


The PID settings can be adjusted here as required or, if necessary, can be reset to factory settings using **RESET PID**.

For details on the PID controller, see the chapter “PID Controller - Basics” and associated subchapters.

If necessary, the value ranges used for the cascaded parameter(s) can be adjusted here.

In the example below, the cascaded parameter *Stirrer* is selected for this purpose in the visual representation, and the input fields for *Minimum* and *Maximum* become visible.



After pressing an input field, the key pad appears for typing in the value (also see the “SETPOINT - Setting the setpoint” section.

After setting the desired cascade, entries are confirmed using **OK**.

10.4.5 TotalFlow

Measures and controls the sum of the volume flows of air (*AirFlow*) and the (optional) second connected gas (*Gas2Flow*).

The mixing ratio of air with a second, optional gas is controlled by the *GasMix* parameter. The controller calculates the setpoint for *AirFlow* and *Gas2Flow* on the basis of the setpoint for *TotalFlow* and *Gasmix*. This allows, for example, the volume flows to be kept constant in the event of a changed gas composition, or the gas composition to be kept constant in the event of a changing volume flow. The measurement value is displayed in L min^{-1}.

The sum of the volume flows, *TotalFlow*, is often used in a cascade for pO_2 control. Cascades for pO_2 control can be configured in the editor menu of the pO_2 parameters.

10.4.6 GasMix

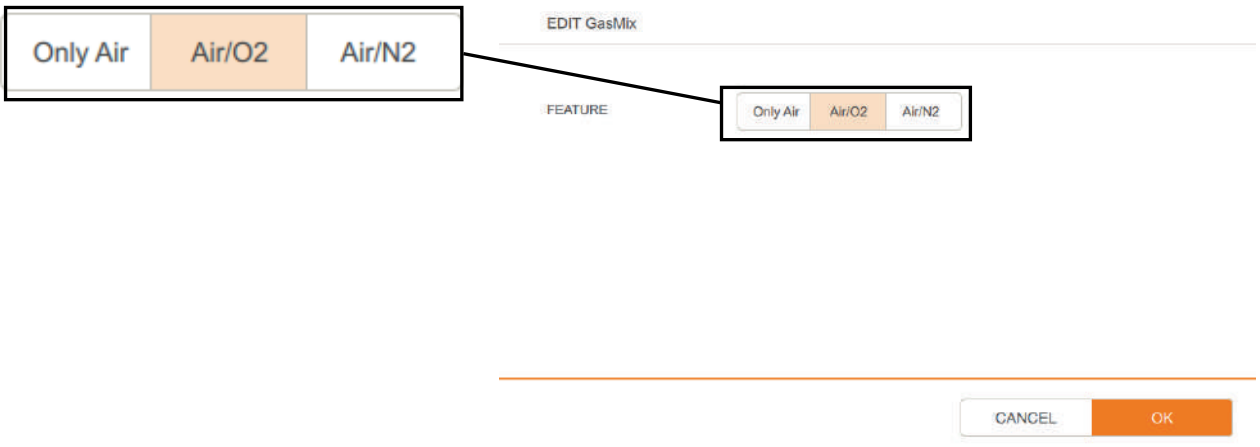
Controls the oxygen concentration in the supply air. This is done by mixing the air and oxygen (O_2) or air and nitrogen (N_2).

INFORMATION

When using oxygen as the second gas, the proportion of oxygen in the gas mixture cannot fall below 20.95 %.

Settings

The optional second gas is configured in the editor menu of the parameter.



The only menu item available here, *FEATURE*, has the following options:

- **Only Air**: Air is exclusively used, with no addition of a second gas. *TotalFlow* therefore remains constant and corresponds to *AirFlow*.
- **Air/O2**: If the setpoint is greater than 21 %, O_2 is additionally mixed in. *TotalFlow* therefore remains constant, the relationship between *AirFlow* and *Gas2Flow* is adjusted automatically.
- **Air/N2**: If the setpoint is less than 21 %, N_2 is additionally mixed in. *TotalFlow* therefore remains constant, the relationship between *AirFlow* and *Gas2Flow* is adjusted automatically.

After selecting the desired option, entry is confirmed using **OK**.

The gas composition *Gasmix* is often used in a cascade for pO_2 control. Cascades for pO_2 control can be configured in the editor menu of the pO_2 parameters.

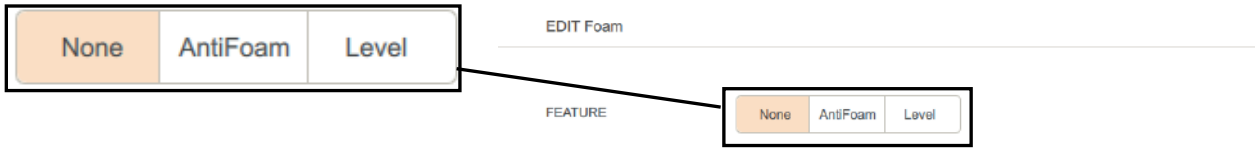
10.4.7 Foam

In the standard setting, measures foam formation (*Antifoam* function) and controls the addition of antifoaming agent from Pump3. The digital antifoam pump is activated as soon as the antifoam sensor comes into contact with foam.

Alternatively, the antifoam sensor can be configured as a level sensor so that Pump3 pumps medium until the desired fill level has been reached again.

Settings

Selection of the foam sensor functions, as well as other possible settings, takes place in the editor menu of the parameter.

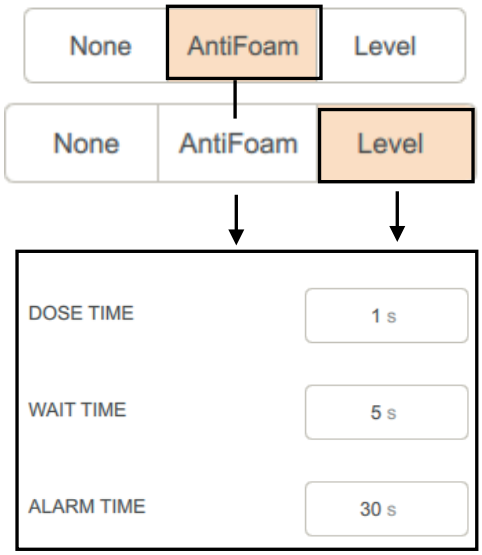


The *FEATURE* menu item has the following three options:

- **None**: No control, foam is only measured

Operation

- **AntiFoam**: Addition of antifoaming agent upon detection of foam
- **Level**: Pump-down of culture medium when a certain fill level is reached



If the *Antifoam* or *Level* function is selected, additional parameter settings are possible:

- **DOSE TIME**: Duration (in seconds) of the addition of antifoaming agent by *Pump3* before a decline in foam is anticipated (*WAIT TIME*).
- **WAIT TIME**: Duration (in seconds) after the addition of antifoaming agent to reduce foam before more antifoaming agent is added.
- **ALARM TIME**: Time (in seconds) after which a parameter alarm is triggered if foam is still detected despite the addition of antifoaming agent.

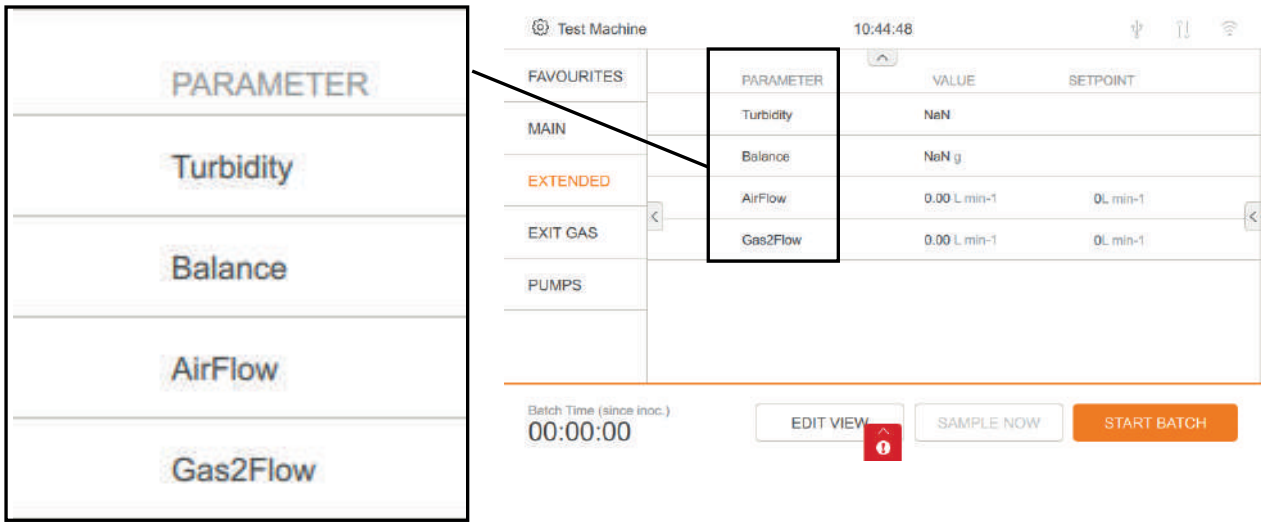
ATTENTION

If the function mode of Pump3 is changed back from *AntiFoam* or *FEED* to *Level*, the pump hoses must be changed because Pump3's direction of rotation remains the same for both functions. Also see the chapters "PUMPS Parameter Group", "Pump3 – Antifoam, Level or Additional Feed Solution" sections

After pressing an input field, the key pad appears for typing in the value (also see the "SETPOINT - Setting the Setpoint" section.

All entries are confirmed with **OK**.

10.5 EXTENDED Parameter Group



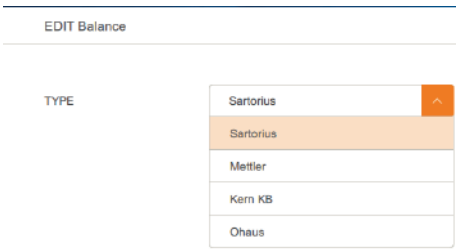
The *EXTENDED* parameter group contains all optional parameters (except exit gas analysis, see *EXIT GAS* parameter group) as well as the standard parameters for the flow rate of air (*AirFlow*).

10.5.1 Turbidity (Optional)

Measures the turbidity in a range from 0 – 4 CU. For more details, see the “Options” section, sub-section “Turbidity Measurement”.

10.5.2 Balance (Optional)

Measures a weight, e.g. a bottle with feed solution. Can be coupled with *Pump4* (feed) to carry out gravimetric feeding. See the “*Pump4* (feed solution)” section for details.



The balance type can be configured in the editor menu of the parameter.

INFORMATION

Balances must be configured with the following values: Baudrate 9600, 8 bits, no parity, 2 stop bits.

For a list of compatible balances or help with the connection, please contact your local INFORS HT service partner.

10.5.3 AirFlow

Measures and controls the flow rate of air into the culture vessel using a mass flow controller (thermal mass meter with control

valve). The measurement system is completely electronic and the measurement value is displayed in L/min.)

INFORMATION

The mass flow controller is calibrated by the manufacturer ex works at standard conditions, i.e. at 1.013 bar and 20 °C. Therefore, for every gas flow rate the standard volume flow is given in L min^{-1}.

The maximum gas flow rate is determined on the basis of the vessel size used in the *VESSEL TYPE* menu. For values, see the main chapter “Technical Data”, chapter “Specifications”, “Gassing”.

INFORMATION

If *TotalFlow* and *Gasmix* are used to control gassing, it is not possible to individually enter setpoints for *AirFlow*. *AirFlow* can only be controlled individually, when *TotalFlow* and *Gasmix* are deactivated.

10.5.4 Gas2Flow

Measures and controls the flow rate of the optional second gas into the culture vessel using a mass flow controller (thermal mass meter with control valve). The measurement system is completely electronic and the measurement value is displayed in L/min.)

In the editor menu of the Gasmix parameter, you can specify whether oxygen (O_2) or nitrogen (N_2) is connected as a second gas or whether no second gas is available.

INFORMATION

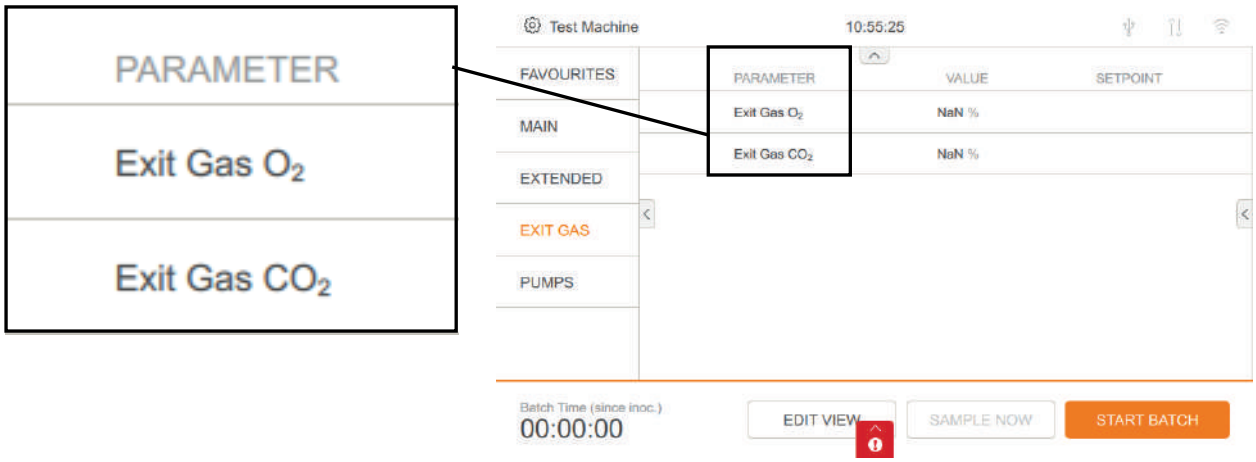
The mass flow controller is calibrated by the manufacturer ex works at standard conditions, i.e. at 1.013 bar and 20 °C. Therefore, for every gas flow rate the standard volume flow is given in L min^{-1}.

The maximum gas flow rate is determined on the basis of the vessel size used in the *VESSEL TYPE* menu. For values, see the main chapter “Technical Data”, chapter “Specifications”, “Gassing”.

INFORMATION

If *TotalFlow* and *Gasmix* are used to control gassing, it is not possible to individually enter setpoints for *Gas2Flow*. *Gas2Flow* can only be controlled individually, when *TotalFlow* and *Gasmix* are deactivated.

10.6 EXITGAS Parameter Group



The *EXITGAS* parameter group contains the parameters for the optional exit gas analysis.

Details on technical data, usage and maintenance requirements for the gas sensors can be found in separate documentation provided by the manufacturer.

ATTENTION

Risk of damage to the gas sensor from operation outside the equipment specifications! The measuring cells of gas sensors based on zirconium oxide (BlueInOne Ferm) are damaged by oxygen-free gas mixtures in continuous operation.

10.6.1 Exit Gas O_2

Measures the oxygen concentration in the exit gas of the bioreactor using a combined BlueInOne Ferm gas sensor (see *Exit Gas CO_2*).

Measurement range: 0 to 50 Vol.% O_2

10.6.2 Exit Gas CO_2

Measures the carbon dioxide concentration in the exit gas of the bioreactor using a combined BlueInOne Ferm gas sensor (see *Exit Gas CO_2*).

Measurement range depends on the chosen variant of the gas sensor:

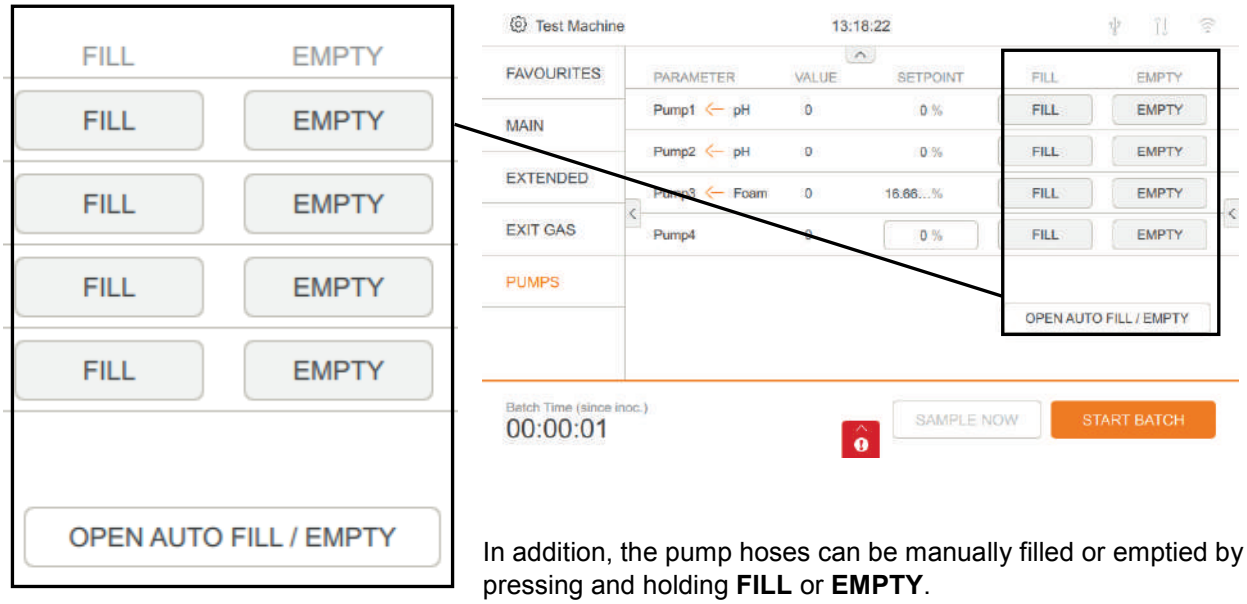
- 0 to 10 Vol. % CO_2

  OR

- 0 to 25 Vol. % CO_2

10.7 PUMPS Parameter Group - General Information

In the *PUMPS* parameter group, the delivery rate of the pumps can be set or monitored and the function mode of the pumps can be configured.



In addition, the pump hoses can be manually filled or emptied by pressing and holding **FILL** or **EMPTY**.

In the *AUTO FILL/EMPTY* sub-menu, there is an option to set a time control for the filling and emptying of every pump.

For details on automatic filling/emptying, see the later chapter “AUTO FILL/EMPTY – Automatically Filling/Emptying Pump Hubes”.

Depending on the function mode, the pumps run in analogue operation with variable speed, or digital operation with a fixed speed.

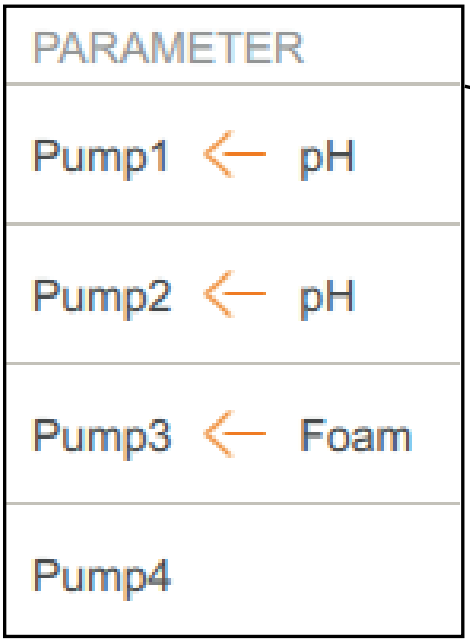
Example

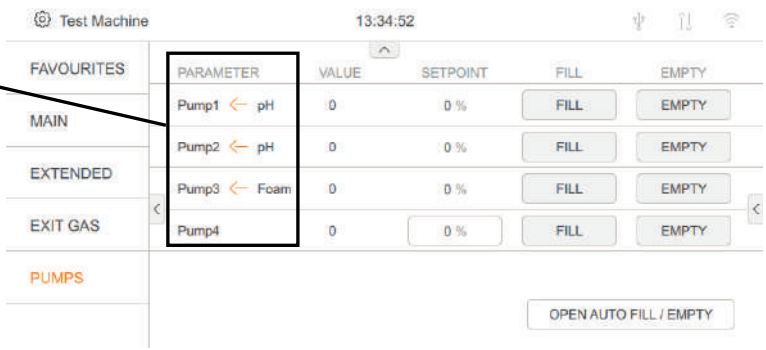
- Analogue: 50 % = half speed = half delivery capacity
- Digital: 50 % = 100 % speed, but only active 50 % of the time = half delivery rate.

Ex works, the pumps are configured as follows:

- **Pump 1:** *ACID* (addition of acid, digital, with fixed speed), controlled by the *pH* parameter.
- **Pump 2:** *BASE* (addition of base, digital, with fixed speed), controlled by the *pH* parameter.
- **Pump 3:** *ANTIFOAM* (addition of antifoaming agent, digital, with fixed speed), controlled by the *Foam* parameter.
- **Pump 4:** *FEED*: Addition of feed solution, analogue, with variable speed, controlled by the user.

The figure below shows the pumps with the factory configuration.





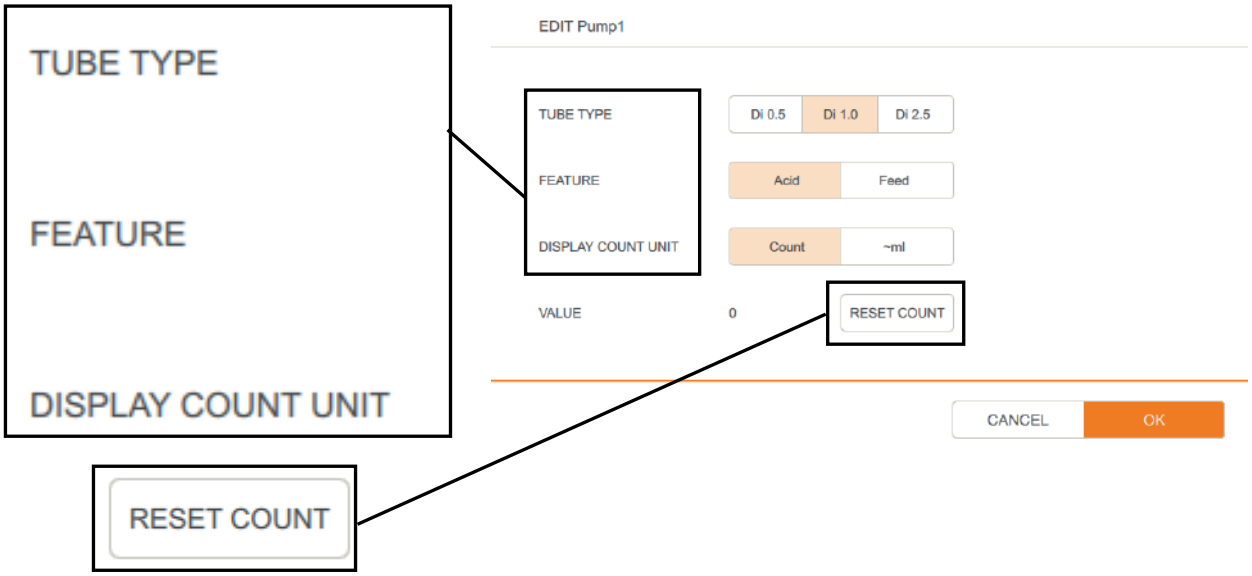
In digital operation, pumping is used as an actuator for other parameters such as *pH* or *Foam* and receive their setpoint from the corresponding controller. This means that it is not possible to enter a setpoint.

When pumping in analogue operation (example to the left, *Pump 4*), setpoints can be specified in % of pump capacity.

The totalled actual value of a pump is displayed, depending on the configuration, in the number of rotations or as an estimated volume in mL or for *Pump4* as a weight in grams in the VALUE column on the main screen.

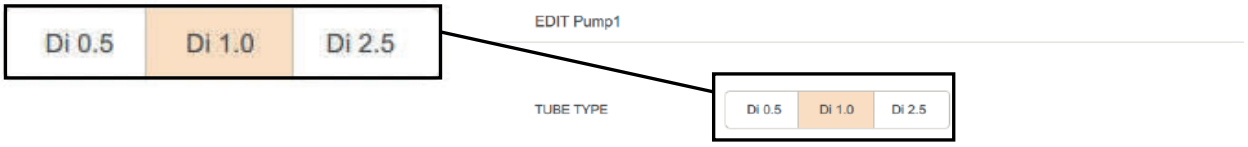
10.7.1 Configuring the Pumps

The editor menu of every pump has four menu items for configuration. The figure below shows the editor menu of *Pump 1* as an example.



TUBE TYPE

Select the pump hose used.



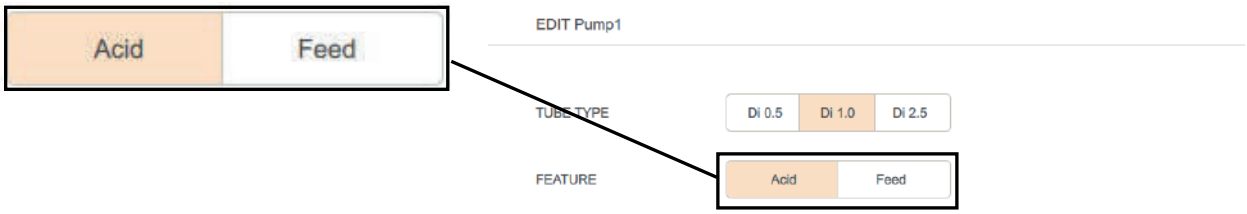
The following pump hoses are available: 0.5 mm, 1.0 mm (standard) or 2.5 mm. On the basis of the selected hose diameter, the pumped volume can be estimated and used for the display of the totalled actual value (selection under *DISPLAY COUNT UNIT*).

INFORMATION

An incorrectly set hose diameter results in an incorrectly totalled actual value.

FEATURE

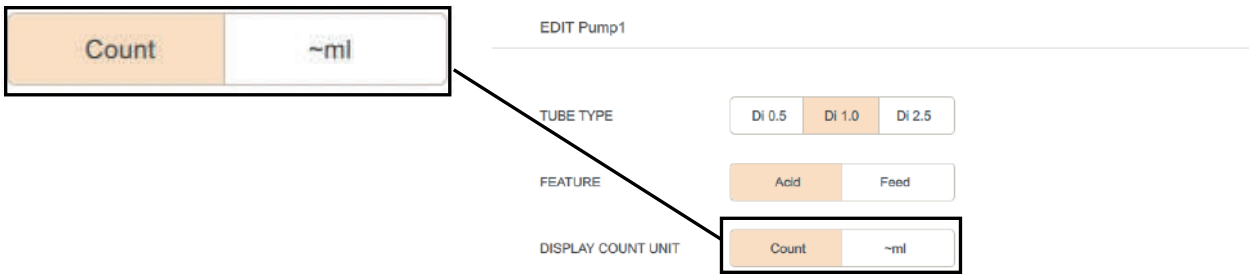
Sets the pump function mode



Since the four pumps have different function modes, they are described in the following sub-sections.

DISPLAY COUNT UNIT

Configures the display of the totalled actual value.



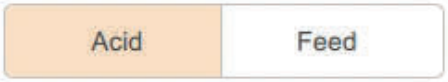
Select either *Count* (number of rotations of the pump head) or *~ml* (the pumped volume estimated on the basis of the hose diameter selected under *TUBE TYPE*).
If a balance (*Balance*) is connected and linked with *Pump4*, *g* (measured pumped weight) is also available at *Pump4*.

RESET COUNT

Here, the totalled actual value of the pump displayed in *VALUE* can be reset to 0.

10.7.2 Pump1 - Acid or Additional Feed Solution

Pump1 can be configured for the *Acid* function mode (factory setting) or *Feed*.



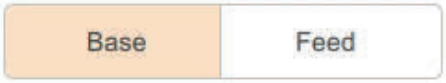
- **Acid**: The pump is operated digitally and is used in pH control to add acid.
- **Feed**: Pump operation is analogue and can be used for addition of another feed solution, for example.

INFORMATION

The function mode of *Pump1* can also be changed by making corresponding entries in the editor menu of the pH parameter.

10.7.3 Pump2 - Base or Additional Feed Solution

Pump2 can be configured for the *Base* operating mode (factory setting) or *Feed*.



- **Base**: The pump is operated digitally and is used in pH control to add base.
- **Feed**: Pump operation is analogue and can be used for addition of another feed solution, for example.

INFORMATION

The operating mode of *Pump2* can also be changed by making corresponding entries in the editor menu of the pH parameter.

10.7.4 Pump3 - Antifoam, Level or Additional Feed Solution

Pump3 can be configured for the *AntiFoam* function mode (factory setting), *Level* or *Feed*.

- **AntiFoam**: The pump is operated digitally, is controlled by the foam sensor (*Foam*) and is used to add antifoaming agent.
- **Level**: The pump is operated digitally, is controlled by the foam sensor (*Foam*) (which is used as a level sensor) and is used to remove culture medium.
- **Feed**: Pump operation is analogue and can be used for addition of another feed solution, for example.

ATTENTION

If the function mode of Pump3 is changed back from *AntiFoam* or *FEED* to *Level*, the pump hoses must be changed because Pump3's direction of rotation remains the same for both functions. See also the "MAIN Parameter Group", "Foam" sections.

INFORMATION

The function mode of *Pump3* can also be changed by making corresponding entries in the editor menu of the *Foam* parameter.

10.7.5 Pump4 - Feed Solution

Pump4 can be configured for the *Feed* function mode (factory setting) or, provided an optional balance is connected and the *Balance* parameter is available, it can be configured for *Balance Feed*.

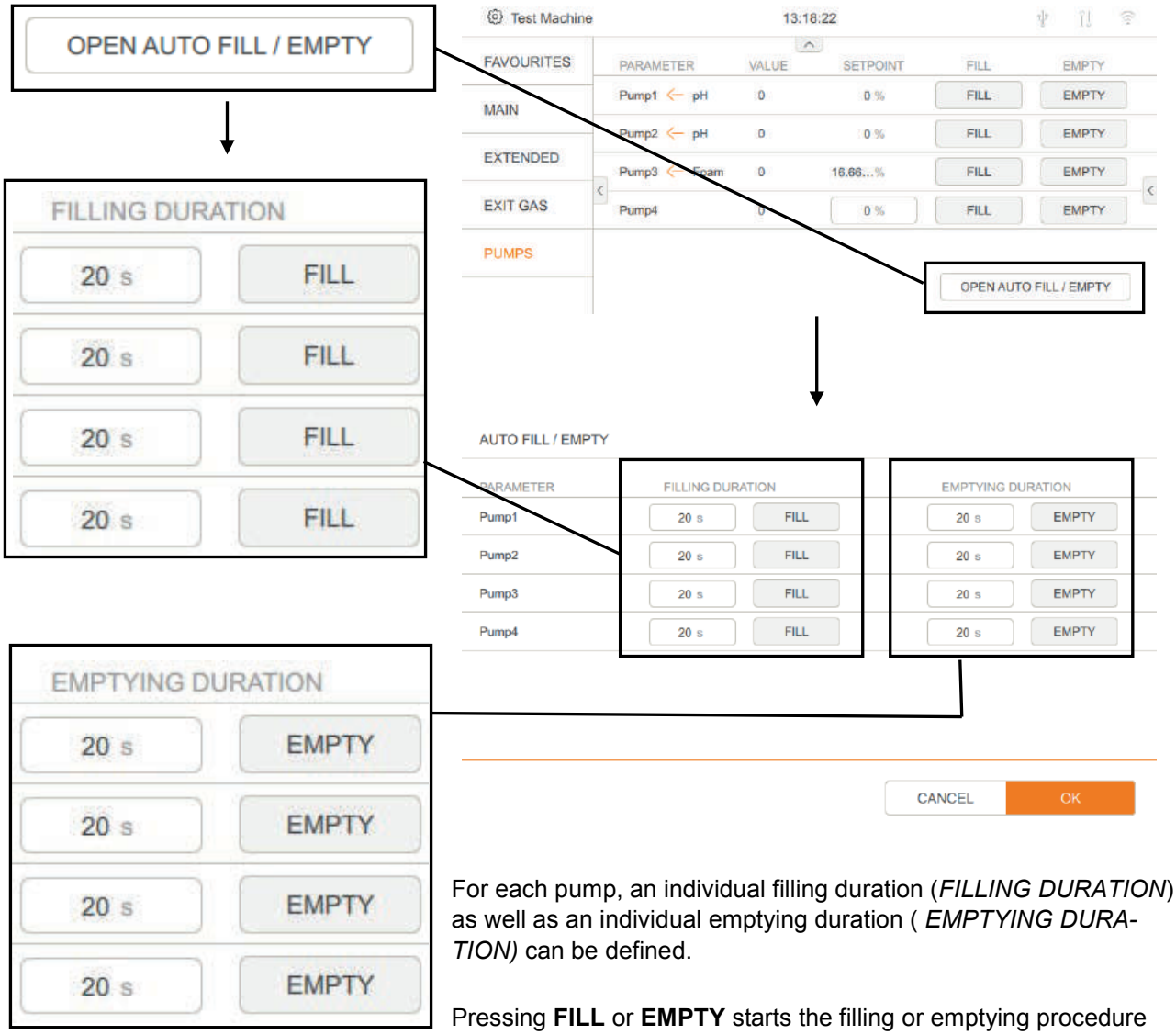
- **Feed**: Pump operation is analogue and is used for the addition of feed solution.
- **Balance Feed**: Pump operation is analogue and is used for the addition of feed solution. The delivery rate is controlled on the basis of the signal of the balance on which the bottle with feed solution is positioned (*Balance* parameter) to guarantee precise dosing.

INFORMATION

In the *Balance Feed* function mode, the additional input fields for adjusting the parameters of the PID controller are available in the editor menu of *Pump4*. For details on the PID controller, see the chapter “PID Controller - Basics” and associated sections.

10.7.6 AUTO FILL/EMPTY – Automatically Filling/Emptying Pump Hoses

Using *OPEN AUTO FILL/EMPTY* in the pump menu, the sub-menu for automatic filling and emptying of the pump hoses is opened.



For each pump, an individual filling duration (*FILLING DURATION*) as well as an individual emptying duration (*EMPTYING DURATION)* can be defined.

Pressing **FILL** or **EMPTY** starts the filling or emptying procedure for the corresponding pump and the pump hsoe is filled or emptied for the set filling or emptying duration.

If a filling or emptying procedure is active, the remaining filling or emptying time is displayed.

In the example to the left, the remaining filling time is displayed.

The menu cannot be closed while at least one filling or emptying procedure is active. The menu can be closed using **OK** as soon as all filling or emptying procedures are completed.

WARNING

If the reagent bottles are not connected correctly, reagents may emerge when filling the pumps and reagent hoses.

10.8 Calibration

Sensors for measuring the pH, pO_2 and turbidity must be calibrated before each cultivation. Depending on the sensor and measurement system, either a 2-point calibration is required, or a 1-point calibration and a zero point adjustment is sufficient.

The various calibrations are described in the following sections.

10.8.1 Calibrating the pH Sensor - General Information

In general for all pH sensors, it is required for a reliable pH measurement that a 2-point calibration with an upper and lower reference buffer is always carried out. Also, the calibration must be carried out again before every cultivation process. The calibration must be carried out before sterilisation, i.e. before installing the pH sensor in the culture vessel.

Detailed information on the calibration of the pH sensor and the pH buffers supported by the sensor can be found in the separate documentation provided by the sensor manufacturer.

INFORMATION

If the pH sensor has already been calibrated before connection to the system, the bioreactor will use this data and calibration on the operating panel is no longer necessary.

The pHs and the temperature dependence of pH buffers are saved in the pH sensors and are automatically detected during calibration. It is therefore not necessary to carry out a separate temperature measurement of the buffer solution.

Refer to the sensor documentation provided by the sensor manufacturer for information on technical data, use, servicing and maintenance.

10.8.2 Calibrating the pH Sensor - Procedure

Proceed as follows to calibrate the pH sensor at the operating panel:

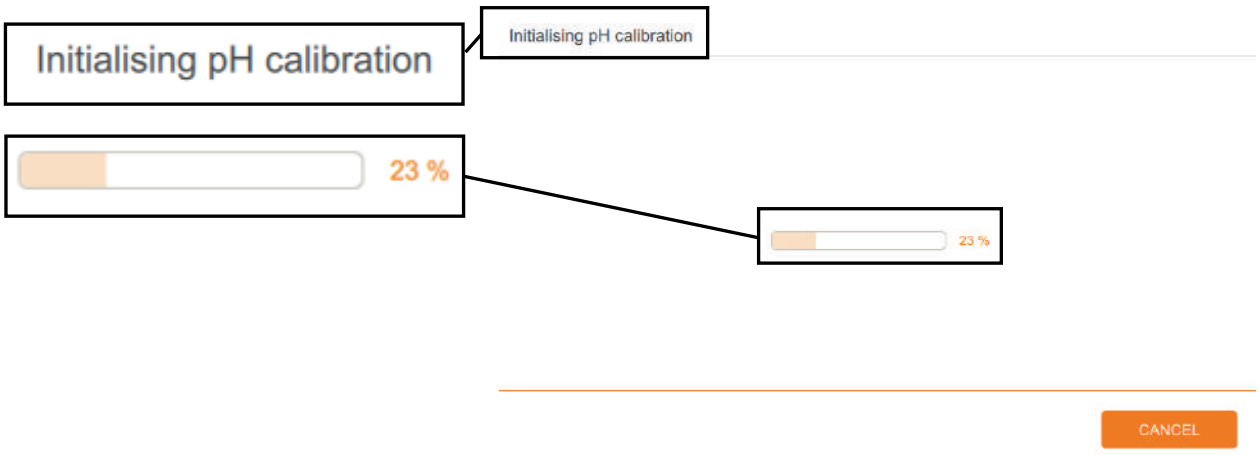
Procedure

1. Connect the sensor cable. For details, see the “Before Cultivation” section and the “Connecting the pH Sensor” sub-section.
2. Carefully remove the cap with storage solution from the pH sensor and rins the sensor with distilled water. Do not rub it.

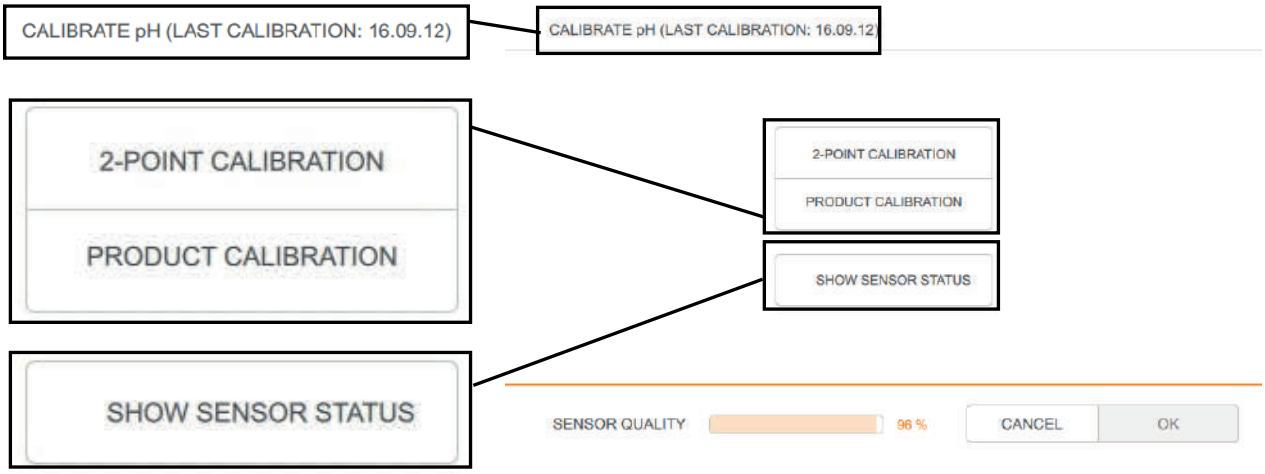
ATTENTION

Wiping or rubbing the pH sensor after rinsing can generate an electrostatic charge. This can greatly increase the response time and generate incorrect measured values. At most, lightly dab the pH sensor after rinsing, **NEVER** wipe or rub.

3. Call up the calibration menu of the pH parameter and wait for the short initialisation phase to complete.



After the initialisation phase, the menu display changes:

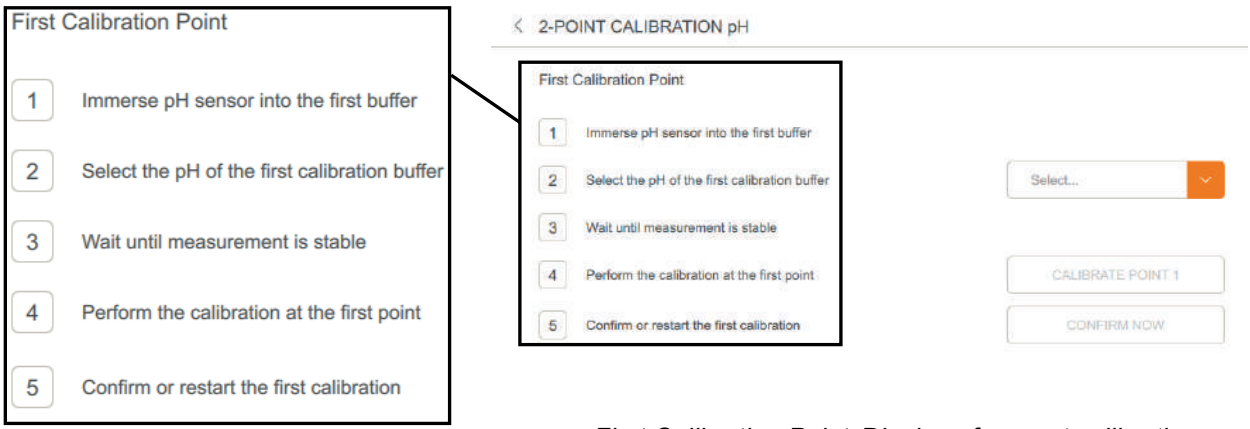


- Header: Date of the last calibration
- **2-POINT CALIBRATION**: Select 2-point calibration
- **PRODUCT CALIBRATION**: Select product calibration (for details, see the “pH product calibration” section).
- **SHOW SENSOR STATUS**: Shows the data and values produced by the firmware of the sensor manufacturer that is integrated in the sensor.

4. Select 2-point calibration.

The menu display changes and displays the following:

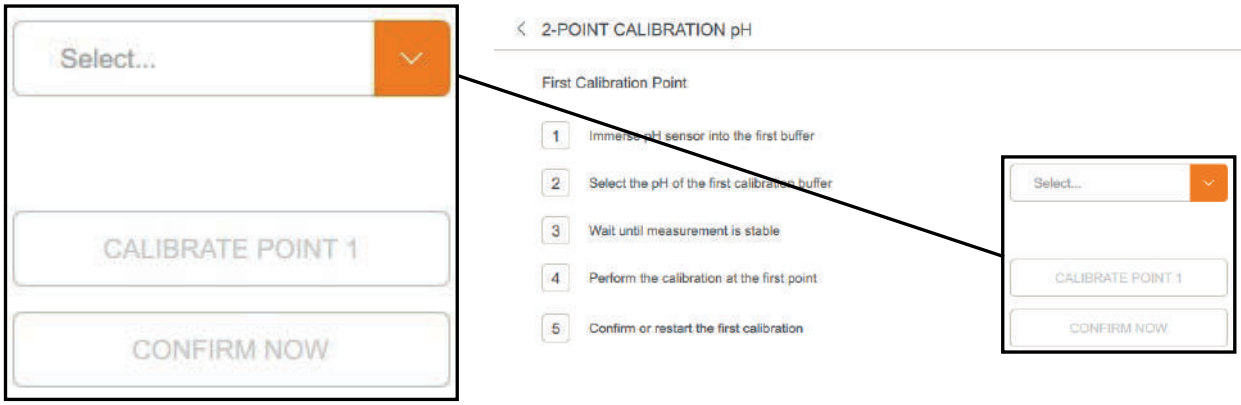
Left-hand side



- *First Calibration Point*: Display of current calibration point (first calibration point)
- 1 *Immerse pH sensor into the first buffer*: Hold the pH sensor in the buffer solution of the first calibration point
- 2 *Select the pH of the first calibration buffer*: Select the pH of the buffer solution for the first calibration point.

- 3 *Wait until measurement is stable*: Wait until the measured value is stable.
- 4 *Perform the calibration at the first point*: Start the calibration procedure for the first calibration point.
- 5 *Confirm or restart the first calibration*: Confirm the calibration or start it again.

Right-hand side



- Pull-down menu for selection of the desired reference value:

INFORMATION

The pH sensors are calibrated with the following standard settings ex works: Buffer pH 4 (calibration point 1) buffer pH 7 (calibration point 2) at room temperature. For calibration with automatic buffer detection (*Auto* in pull-down menu), the following buffers are defined ex works: pH 4.01, pH 7.00 and pH 10.01.

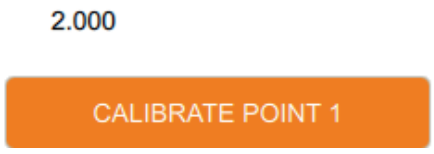
- **CALIBRATE POINT1**: Start the calibration procedure for the 1st reference.
- **CONFIRM NOW**: Confirm the calibration and continue with the 2nd reference

5. Hold the pH sensor in the buffer solution of the first calibration point, e.g. solution of pH 4.01.

6. In the pull-down menu, either select the *Auto* entry or the appropriate value of the possible pH reference value.

INFORMATION

The pH sensors are calibrated with the following standard settings ex works: Buffer pH 4 (calibration point 1) buffer pH 7 (calibration point 2) at room temperature. For calibration with automatic buffer detection (*Auto* in pull-down menu), the following buffers are defined ex works: pH 4.01, pH 7.00 and pH 10.01.



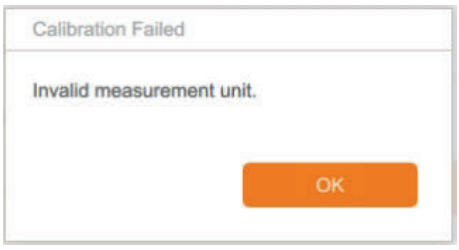
When the desired reference value has been selected, the current pH measured value appears and **CALIBRATE POINT 1** is activated, i.e. the button turns orange.

7. Press **CALIBRATE POINT 1** to begin calibration.

CONFIRM NOW creates a visual representation of the progress of the calibration procedure and slowly turns orange. The procedure takes 3 minutes to guarantee a stable value. When the countdown has finished, CONFIRM NOW is completely coloured in.

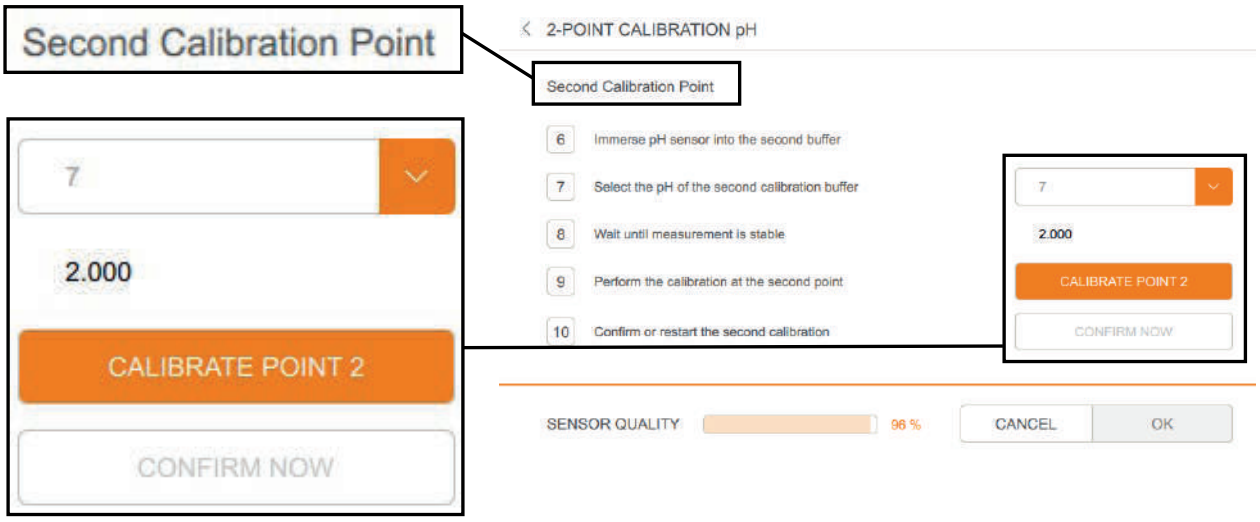
INFORMATION

If the measured value is assumed to be already stable, the waiting time can be skipped by pressing CONFIRM NOW to continue with the second calibration point.



In the event of a problem, an error message is displayed with a corresponding notice.

If the calibration was successful, the menu display changes automatically to calibrate the second point.



8. Rinse the pH sensor with distilled water. Do not rub it.
9. Hold the pH sensor in the buffer solution of the second calibration point, e.g. a solution of pH 7.00.
10. In the pull-down menu, either select the *Auto* entry or the appropriate value of the possible pH reference value.

    For information on the selectable reference value, see calibration point 1.

    When the desired reference value has been selected, the current pH measured value appears and **CALIBRATE POINT 2** is activated, i.e. the button turns orange.
11. Press CALIBRATE POINT 2 to begin calibration.

    CONFIRM NOW creates a visual representation of the progress of the calibration procedure and slowly turns orange.

    The procedure takes 3 minutes to guarantee a stable value.

    When the countdown has finished, CONFIRM NOW is completely coloured in.

INFORMATION

To skip the waiting time, the same conditions apply as described for the first calibration point.

12. If the calibration was successful, confirm it with **OK** and leave the menu.

10.8.3 pH Sensor Product Calibration

It is possible to adjust the calibration curve to the current process conditions using product calibration. This could be necessary if there is a possibility of drift of the displayed pH during a long-term cultivation, for example.

INFORMATION

Product calibration can only be carried out and is only effective if the externally measured and entered pH value does not deviate from the original pH value by more than 2 pH units.

Proceed as follows for a product calibration:

Procedure

1. Call up the calibration menu of the pH parameter and wait for the short initialisation phase to complete.

INFORMATION

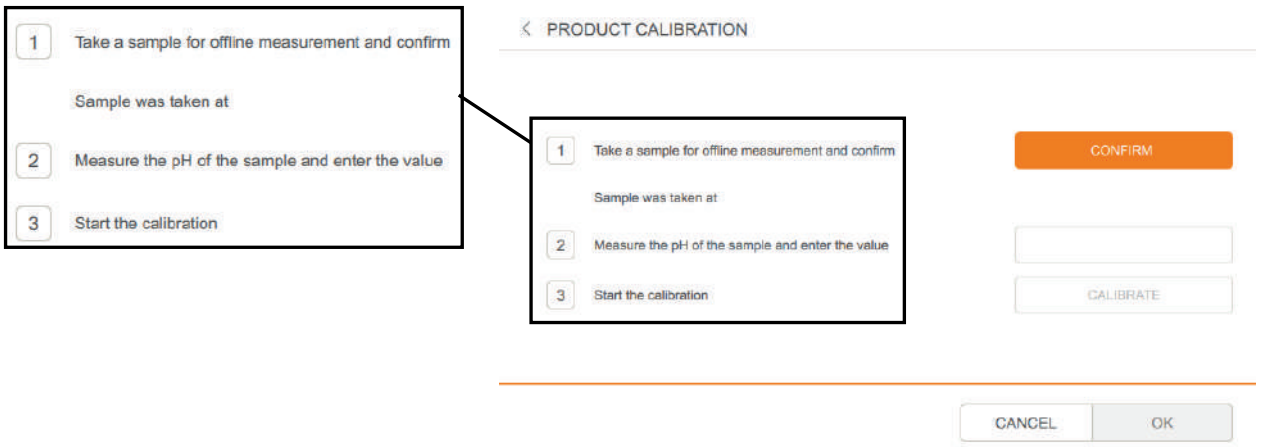
The various menu displays of the initial calibration menu are not displayed in detail in this section, they are shown in “Calibrating the pH Sensor - Procedure”.

2. In the menu display that follows the initialisation phase, press **PRODUCT CALIBRATION**

The menu display changes and now displays the following:

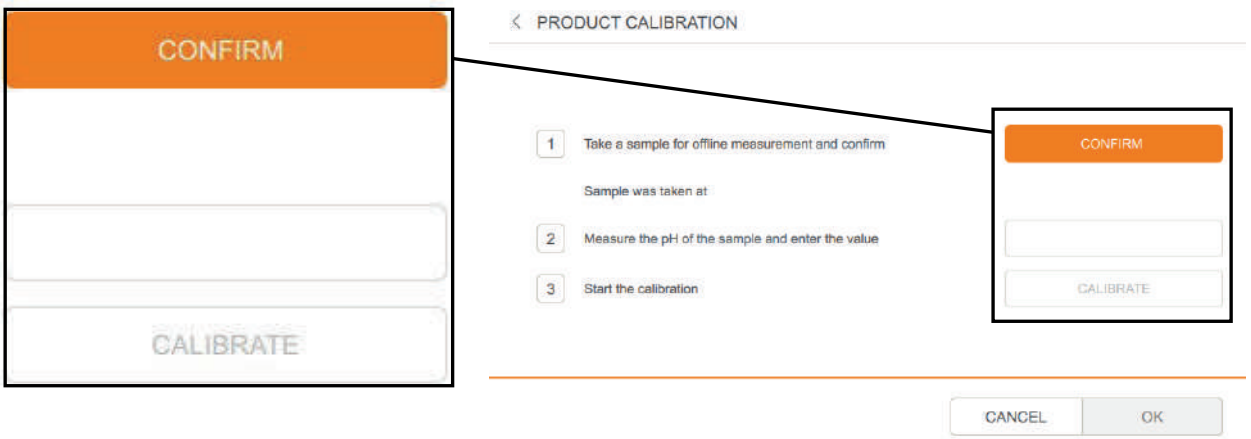
Operation

Left-hand side



- 1 *Take sample for offline measurement and confirm:* Take a sample for external measurement and confirm.
- *Sample was taken at*: Display of date and time of the sampling.
- 2 *Measure the pH of the sample and enter the value:* Measure the pH of the sample and enter the value.
- 3 *Start the calibration:* Start the calibration.

Right-hand side



- **CONFIRM**: Confirm the sampling, generate a time stamp.
- Empty input field: Enter the externally measured pH value of the sample.
- **CALIBRATE**: Start product calibration.

3. Take a sample from the process (in the culture vessel).

   There are two possible approaches:

   a) Confirm the sampling (generate a time stamp), carry out a laboratory measurement of the pH value for the sample, enter the measured value and carry out product calibration.

      OR:

   b) Confirm the sampling (generate a time stamp), leave the calibration menu and carry out the product calibration with an external measured value at a later time.

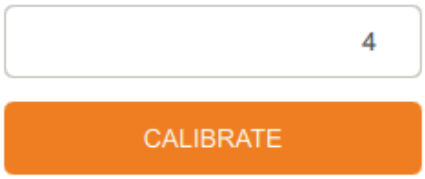
Variant a)

Procedure

1. Press **CONFIRM**.

   The date and time of the sampling are now displayed.

2. Carry out a laboratory measurement of the pH value for the sample.

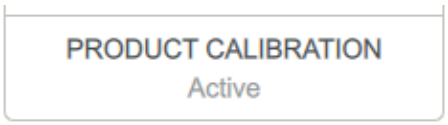


3. Enter the measured pH value of the sample, in the example to the left, pH 4.

4. Press **CALIBRATE** to begin calibration.

5. Wait until the calibration is complete.

6. Confirm the calibration with **OK** and leave the menu.



In the calibration menu, Active is displayed under PRODUCT CALIBRATION to show that a product calibration was carried out and is active.

INFORMATION

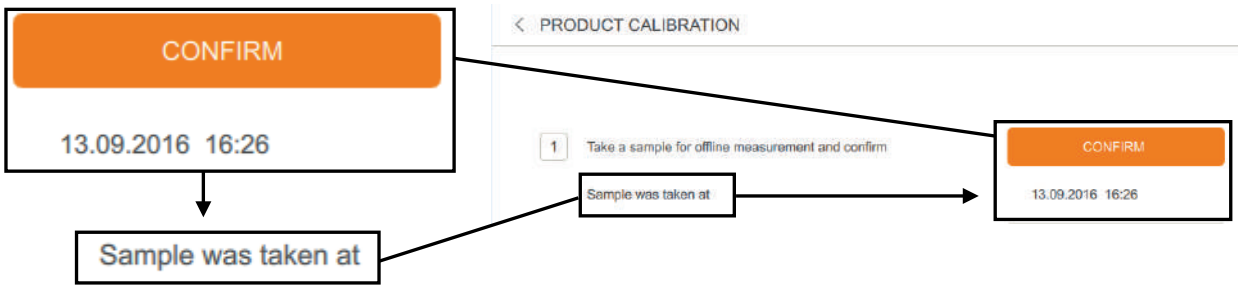
The original calibration curve can be restored again using a 2-point calibration. 2-point calibration cancels the product calibration.

Variant b)

Procedure

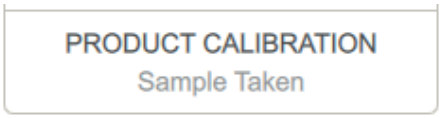
1. Press **CONFIRM**.

   The date and time of the sampling are now displayed.



2. Leave the calibration menu using **OK** and carry out a laboratory measurement of the pH value for the sample at a later time of your choosing.

   In the calibration menu, Sample Taken is displayed under PRODUCT CALIBRATION to show that sampling was carried out but product calibration is not yet active.



3. To carry out product calibration, proceed as in Variant a) from step 3.

10.8.4 Calibrating the pO_2 Sensor - General Information

Generally speaking, the following applies: The pO_2 sensor should be calibrated after autoclaving has been performed because the sterilisation process may change the slope of the pO_2 sensor response.

As a rule, a 1-point calibration to 100 % is usually sufficient for exact measurement, and should be carried out before each cultivation.

The zero point (0 % calibration) should be checked at regular intervals of approximately 6 months.

When carrying out a 2-point calibration, the 0 % calibration must be carried out before the 100 % calibration.

Detailed information on calibration, general use, service and maintenance can be found in the separate documentation provided by the sensor manufacturer.

INFORMATION

pO_2 sensors are preconfigured by the equipment manufacturer to the measured value %-sat. Replacement sensors must also be configured by the equipment manufacturer.

Conditions for 100 % calibration

The 100 % calibration of the pO_2 sensor described here is carried out under the following conditions:

- in medium
- at operating temperature
- at the maximum expected stirrer speed
- at the maximum expected gas flow rate for air or the oxygen-containing gas(ses) provided for the cultivation
- at the expected pH

Conditions for 0 % calibration

The operating conditions for 0 % calibration are the same as for 100 % calibration. Gassing is one exception.

To displace the oxygen from the medium, the medium has to be gassed with nitrogen instead of air or the gas(ses) used for cultivation before and during 0% calibration.

10.8.5 Calibrating the pO_2 sensor - Procedure

The following example describes a 2-point calibration. The correct sequence should be observed. This means that the zero point (0 %) is calibrated before 100% oxygen saturation.

Gassing with nitrogen takes place for the 0 % calibration; 100 % calibration takes place with air.

INFORMATION

A pO2 value of -1.0 indicates an error in communication.

Operation

Proceed as follows:

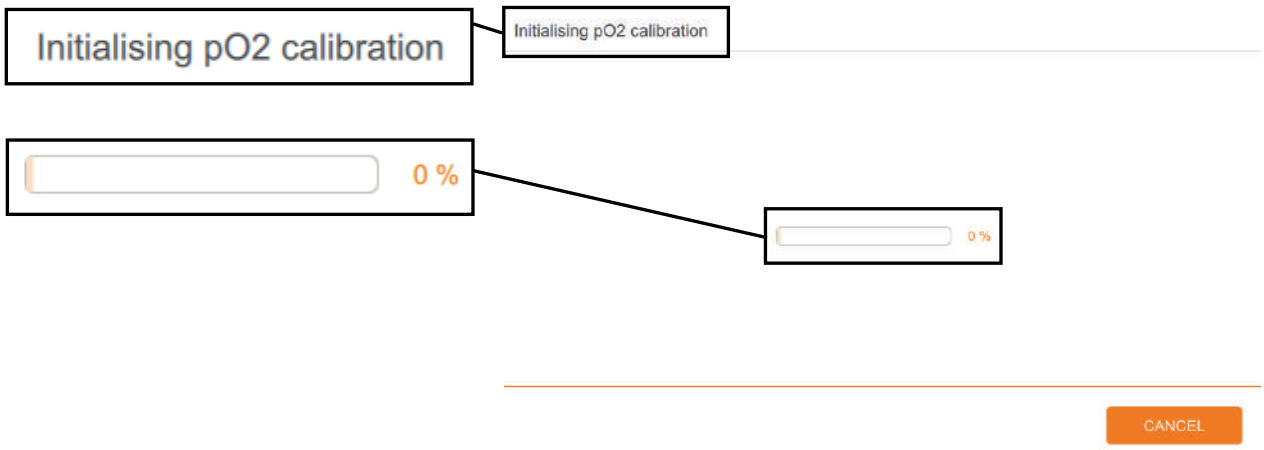
Procedure

1. Connect the nitrogen to the gassing line (O_2/N_2 IN connection). In the process, leave the gas supply closed.

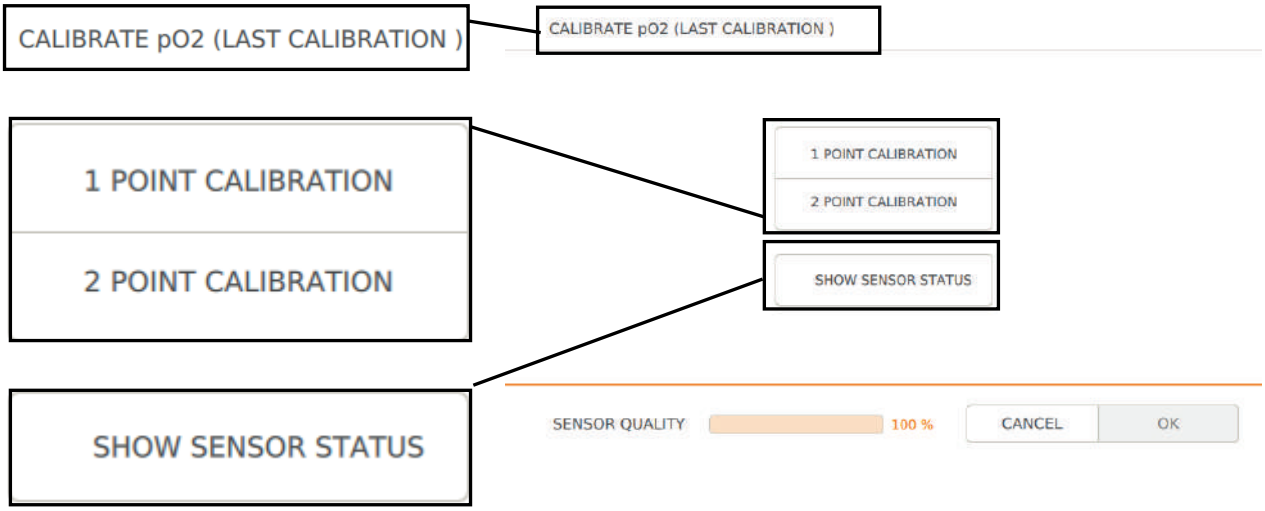
INFORMATION

If necessary, enter setpoints for temperature and pH, activate parameters and press **START BATCH** and wait until the desired operating temperature and the expected pH have been reached.

2. Call up the calibration menu of the pO_2 parameter and wait for the short initialisation phase to complete.



After the initialisation phase, the menu display changes:



- Header: Date of the last calibration
- **1 POINT CALIBRATION**: Select 1-point calibration
- **2 POINT CALIBRATION**: Select 2-point calibration
- **SHOW SENSOR STATUS**: Shows data and values produced by the firmware of the sensor manufacturer integrated in the sensor.

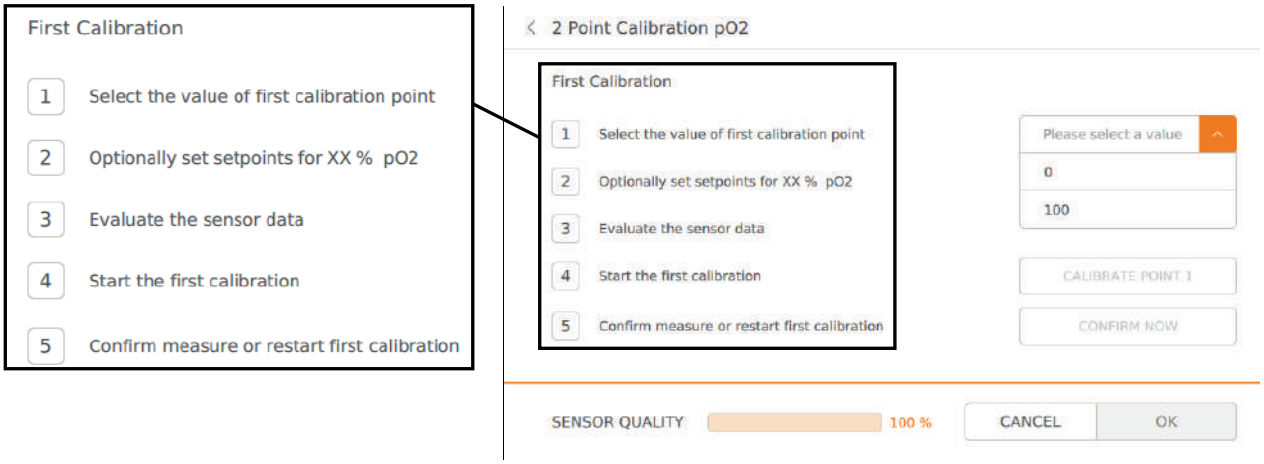
3. Select 2-point calibration.

INFORMATION

2-point calibration can also be carried out in 1-point calibration mode one point after the other.

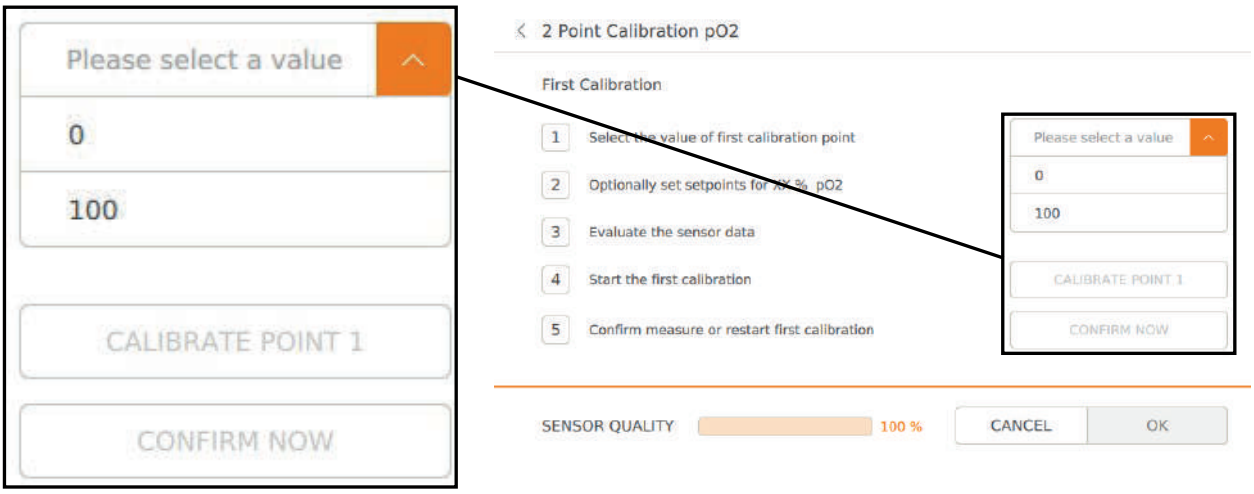
The menu display changes and displays the following:

Left-hand side



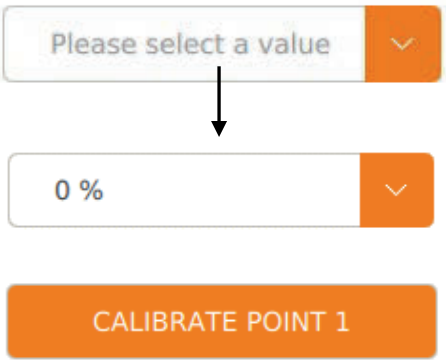
- *First Calibration*: First calibration point
- 1 *Select the value of first calibration point*: Select the reference value of the first calibration point
- 2 *Optionally set setpoints for XX % pO2*: If necessary, enter the setpoint for XX % pO_2 (corresponding gassing and stirrer are activated by this)
- 3 *Evaluate the sensor data*: pO_2 measuring display
- 4 *Start the first calibration*: Start the calibration procedure for the first calibration point
- 5 *Confirm measure or restart first calibration*: Confirm the measurement or start the calibration again.

Right-hand side



- Pull-down-menu for the selection of 0 % or 100 % calibration point.
- Input field for the desired pO_2 setpoint in % (depending on the input, activates the corresponding gassing with air/nitrogen and the stirrer)
- pO_2 measuring display
- **CALIBRATE POINT1**: Start the calibration procedure for the 1st calibration point.
- **CONFIRM NOW**: Confirm the calibration and continue with the 2nd calibration point

4. Slowly open the nitrogen supply.



5. In the pull-down-menu, select the value 0 (%).

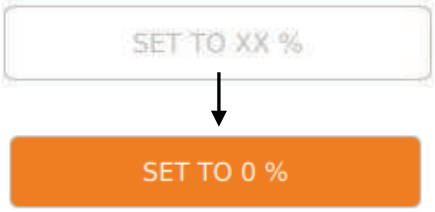
The selection is displayed.

CALIBRATE POINT 1 is activated, i.e. the button turns orange.

INFORMATION

This facilitates a calibration of the sensor outside of the medium, i.e. without active gassing for a standard calibration outside the medium, which is not described here.

Operation



6. Enter the setpoint 0 (%) in the input field.

   The input field turns orange and displays the set setpoint.

A dialogue appears with the notice *Make sure that N2 is connected to the Gas2 connector*
Make sure that N2 is connected to the correct connection.

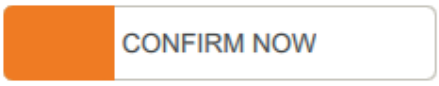
7. If necessary, connect the nitrogen and confirm with **OK**.

   Gassing with nitrogen is activated, the stirrer switches on simultaneously.

8. Wait until the oxygen has been displaced from the medium, i.e. wait until the measured value (display via CALIBRATE POINT 1) is stable.

9. Press CALIBRATE POINT 1 to begin calibration.

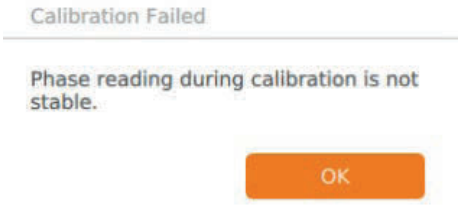
   Gassing and the stirrer are stopped.



CONFIRM NOW creates a visual representation of the calibration procedure and slowly turns orange.
The procedure takes 3 minutes to guarantee a stable value.
When the countdown has finished, CONFIRM NOW is completely coloured in.

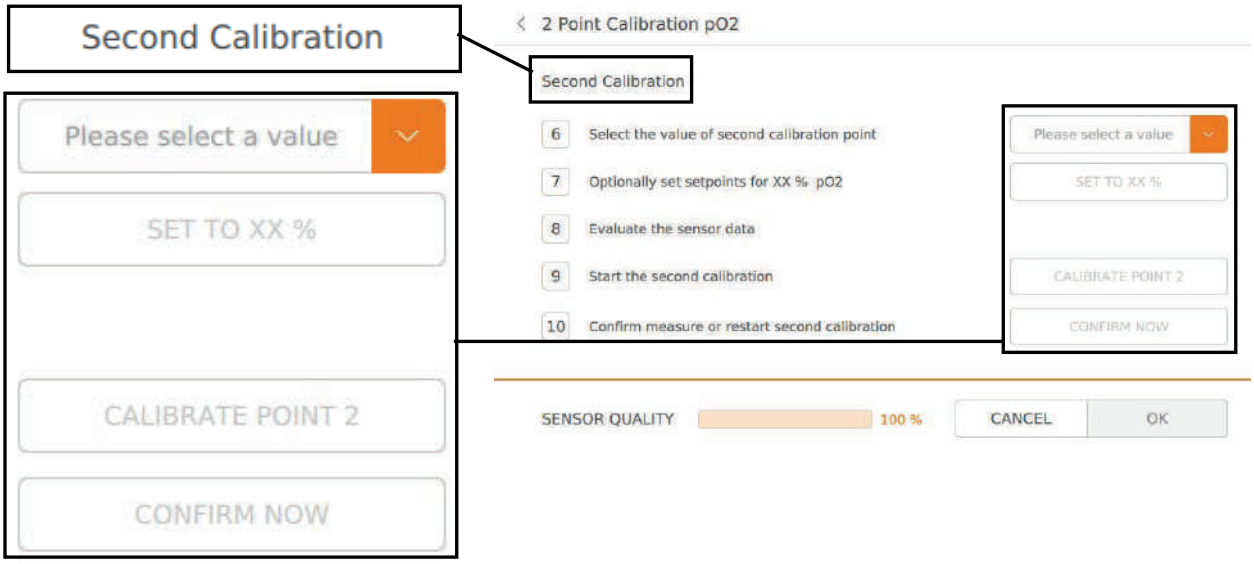
INFORMATION

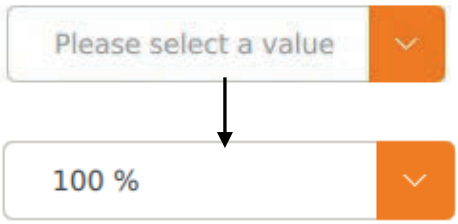
If the measured value is assumed to be already stable, the waiting time can be skipped by pressing CONFIRM NOW to continue with the second calibration point.



In the event of a problem, an error message is displayed with a corresponding notice.

If the calibration was successful, the menu display changes automatically to calibrate the second point.





10. In the pull-down-menu, select the value 100 (%).

The selection is displayed.

CALIBRATE POINT 2 (or 1, if not calibrated one after the other in 1-point calibration mode) is activated, i.e. the button turns orange.

INFORMATION

This facilitates a calibration of the sensor outside of the medium, i.e. without active gassing for a standard calibration outside the medium, which is not described here.

11. Enter the setpoint 100 (%) in the input field.

The input field turns orange and displays the set setpoint. Gassing with nitrogen is activated (if connected!), the stirrer switches on simultaneously.

12. Wait until the medium is saturated with oxygen, i.e. wait until the measured value (display via CALIBRATE POINT 2) is stable.
13. Press **CALIBRATE POINT 2** (or 1) to begin calibration.

    Gassing and the stirrer are stopped.

CONFIRM NOW creates a visual representation of the calibration procedure and slowly turns orange.
The procedure takes 3 minutes to guarantee a stable value.
When the countdown has finished, CONFIRM NOW is completely coloured in.

INFORMATION

To skip the waiting time, the same conditions apply as described in the first calibration point.

14. If the calibration was successful, confirm it with **OK** and leave the menu.

10.8.6 Calibrating the Turbidity Sensor - General Information

Optek turbidity sensors (optional) are pre-calibrated in the factory. Inserts are available for reference measurement.

Due to the different light absorption of different media, zero point calibration of the turbidity sensor should be performed before each cultivation process. This can be done either **before or after** sterilising, depending on the application in question.

Conditions for zero point calibration of the sensor

The sapphire windows of the optical density sensor must be clean and free of air or gas bubbles.

The light absorption of the medium before activation of the gassing and before inoculation can be used as a reference value for the zero point.

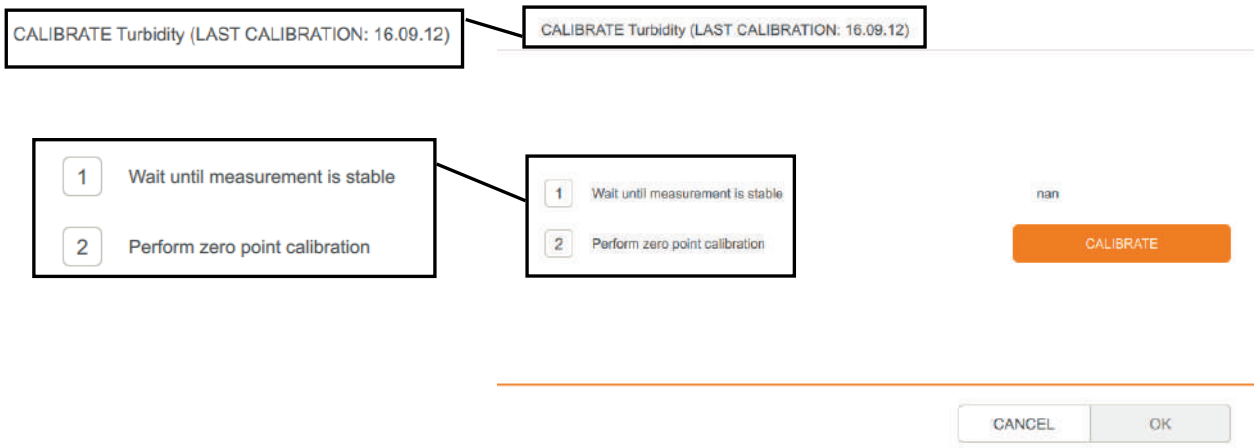
10.8.7 Calibrating the Turbidity Sensor - Procedure

Proceed as follows to calibrate the zero point of the (optional) turbidity sensor:

Procedure

1. Connect the sensor cable.
2. Call up the calibration menu of the *Turbidity* parameter.

In the header, the menu displays the date of the last calibration, and the calibration sequence appears to the left:

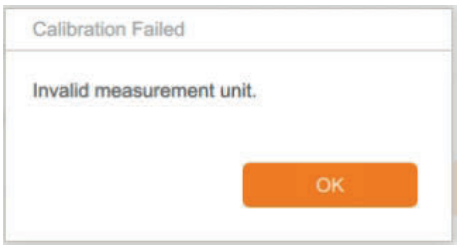


- *1 Wait until measurement is stable*: Wait until the measured value is stable.
- *2 Perform zero point calibration*: Carry out zero point calibration.

3. Wait until the measured value is stable (display via **CALIBRATE**).

4. Press **CALIBRATE**.

If the calibration was successful, **OK** is activated and can be pressed to confirm the calibration and leave the menu.



In the event of a problem, an error message is displayed with a notice.

10.9 PID Controller – Basic Principle

PID controllers (*Proportional Integral Derivative* controller) are used for some of the parameters. The PID function is based on a generic formula provided as example:

$$Error_n = \frac{Set - Act}{Max.Value - Min.Value}$$

$$Output_n = P.Term * \left\{ Error_n + I.Term \cdot \int_{i=0}^{n} Error_i + D.Term \cdot (Error_n - Error_{n-1}) \right\}$$

Explanation of the formula

- Error = deviation between setpoint value and actual value.
- P = proportional factor, proportional response to an error, used to reach a setpoint.
  The bigger the value, the sharper the control.
- I = integral factor, integration of the error in 1/second.
  A typical integral factor is < 0.05.
- D = differential quotient, derivative of the error, set in seconds (mostly to 0).

Be aware of the following relating to the individual factors:

Proportional factor

The change of the proportional factor has a considerable effect on a running process.

If the proportional factor is increased excessively, this causes oscillations in the control loop around the setpoint value.

Example, the pH parameter

To achieve the setpoint value, a little acid, then a little base, acid again, then base etc. is added.

If the proportional factor is reduced excessively, the controller hardly reacts to the deviations and never achieves the setpoint value.

Integral factor

The integral factor should have a low value and only be changed a little in small steps with long pauses.

The ideal approach is to switch off the equipment briefly after changing the integral factor in order to delete the pending error calculation.

A typical integral factor is < 0.05. It should equate to the reciprocal value of double to quadruple the system's cycle duration. The higher the entered value, the less the time (in seconds) remains for control.

A higher value than 0.05 is generally of no use as it exceeds the time minimum for which the control is required. This causes fluctuations in the control circuit.

Example of calculation of the integral factor

The cycle duration of system oscillations is measured at 50 seconds from amplitude to amplitude. The integral factor is thus calculated as follows:

1 / (50 sec x 2) = 0.01 1/sec

1 / (50 sec x 4) = 0.005 1/sec

Integral factor	Seconds
01	10
0.05	20
0.001	100
0.005	200

Differential quotient

The differential quotient is rarely required. It is set to 0 (zero) at the beginning.

A high value is only necessary if major changes are made in quick succession. In all circumstances it causes the controller output to react stronger.

10.9.1 Table with Setting Values for PID Controller

Setting value	Description
P (Prop. Term)	Proportional factor: The greater the discrepancy between the set-point value and the actual value the greater the controller output
I (Integ. Term [1/s])	The integral factor aggregates all errors over the time. If the setpoint is not achieved using the proportional factor, the integral factor adjusts the output successively until the setpoint value is achieved. An integral factor set too high will lead to oscillation of the control loop.
D (Diff Term [s]	The differential quotient calculates the change in the actual value over the time and counteracts this change to limit any overshoot.
Neg. Factor	The negative factor can be used to add weighting to two-sided control (+100 to -100 %) (e.g. heavy acid, light alkali). In the process 1 is the balance and 0.5 or 2 equate to the half or double the controller output accordingly. Example: Nitrogen influences the pO_2 value less than oxygen, thus a negative factor of 2 can compensate for the reaction of the controller.
Deadband	If a dead band is entered, no control is implemented within this value at either side of the setpoint value (symmetrically, + / -). I.e. the controller output is = 0. The dead band is used for pH control.
I Limit (Integ. Limit [%])	The integral influence is used to ensure that the integral factor cannot increase over an indefinite period. This limits erroneous accumulation. The integral influence is set between 0 and 100 % of the controller output.
Eval Time [s]	The evaluation time determines the intervals in seconds at which the PID value is recalculated. The controller speed is defined this way. A scanning time of 10 seconds is a good average value.

10.9.2 Useful Information for Changing PID Controller Settings

To adjust the PID controller settings, proceed as follows:

Procedure

1. For readjustment of a PID controller, start with the setting for the proportional factor. Select a proportional band width as large as possible.
2. Reset the integral factor and the differential quotient to zero.
3. Increase the proportional factor until the controller causes the actual value to oscillate.
4. Measure the oscillation duration, e.g. with the bioprocess platform software eve® from the manufacturer.
5. Halve the proportional factor and vary the integral factor between the reciprocal value of the doubled and quadrupled oscillation duration.

10.9.3 Adjusting PID Settings

ATTENTION

Inappropriate changes to the PID controller settings may have a negative effect on the fermentation/cultivation process and cause loss of property.

Therefore, only change factory settings of the PID controller(s), if you are fully aware of the consequences or after consulting the manufacturer!

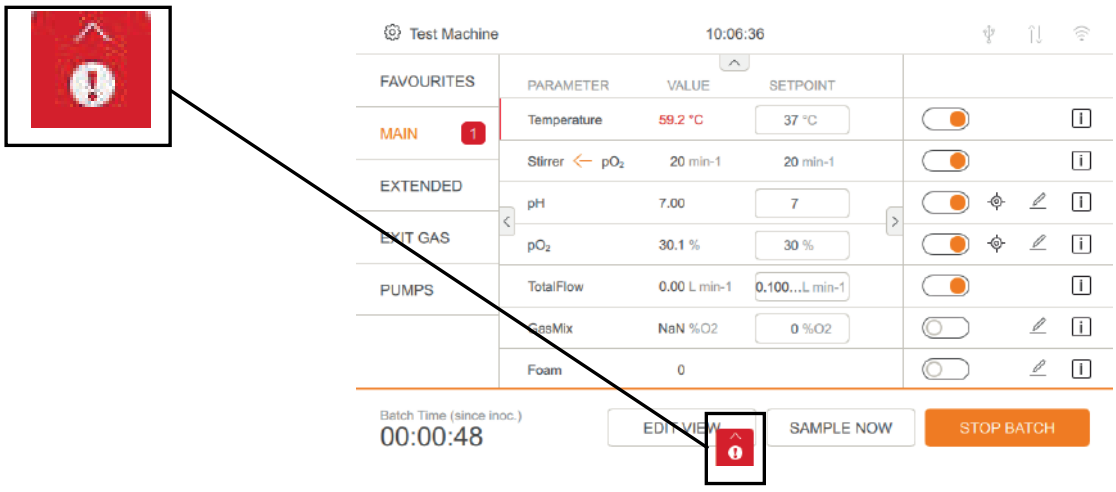
PID control may be configured for parameters *pH*, *pO_2 and Pump4* (Function *Balanced Feed*). This is done in the editor menu of the corresponding parameter and therefore described there.

10.10 Alarms – Equipment Alarm Menu

There are two types of alarm that appear in the *Equipment Alarm* menu:

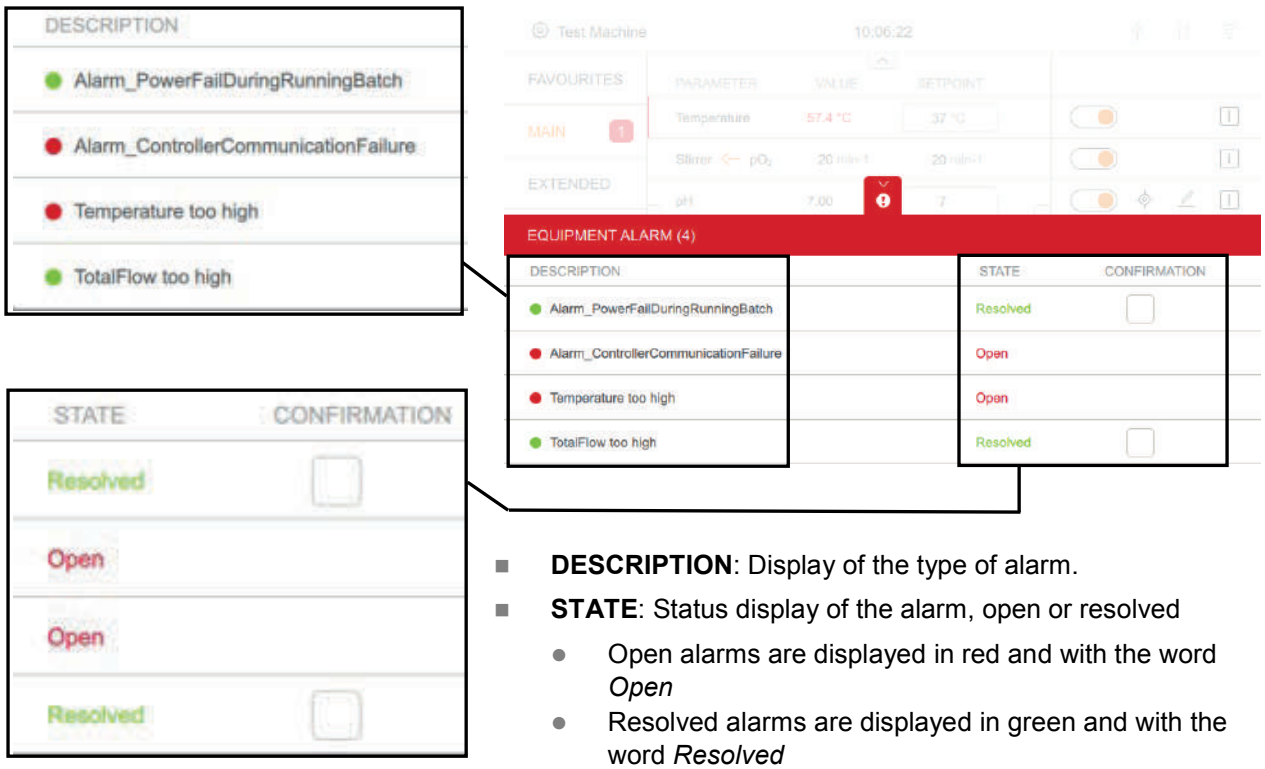
- **Parameter alarms** Display of deviations from actual values and setpoints for parameters after a predefined waiting time. Also see the “Parameter alarms” section.
- **Equipment errors:** If equipment errors occur repeatedly or cannot be resolved, inform an authorised INFORS HT service partner. Also see the fault table in the “Interferences” section.

The *Equipment Alarm* menu is only available when there are open or unconfirmed alarms. Otherwise the alarm symbol (a red exclamation mark highlighted in white on a red background) is hidden in the lower screen edge.



Operation

Pressing the alarm symbol or swiping upwards opens the *Equipment Alarm* menu.



- **DESCRIPTION**: Display of the type of alarm.
- **STATE**: Status display of the alarm, open or resolved
  - Open alarms are displayed in red and with the word *Open*
  - Resolved alarms are displayed in green and with the word *Resolved*
- **CONFIRMATION**: Confirms the alarm and deletes it from the list. The entry in the batch log remains.

11 Cleaning and Maintenance

The following sections describe in detail how the culture vessel and accessories and the basic unit are cleaned and, as required, stored.

In addition, the section contains a maintenance plan and corresponding descriptions for the procedures to be performed by the operator.

11.1 Cleaning Agent and Disinfectant

Intended use	Allowed products/tools
Culture vessel	Water and a non-scratch, non-abrasive sponge or washing-up brush; lab washer with special washing agent (for industry and lab use)
Cleaning agent for denaturation of proteins (e.g. exit gas cooler)	0.1 N NaOH
Cleaning agent for smaller component parts (e.g. exit gas cooler, dip tube)	Ultrasonic bath
Cleaning agent for surfaces	Water
Disinfectant for surfaces	Ethanol, 70 %
Decalcifier for the equipment	Phosphoric acid or citric acid (max. 5 %)

11.2 Cleaning the Culture Vessel - Routine Cleaning

The culture vessel and accessories can be cleaned as soon as they have cooled down after autoclaving.

ATTENTION

Household washing-up liquid and soap (in particular cream soaps) can collect in glass pores and impair later cultivations.

Never clean culture vessels and accessories with household soap and use special cleaning agent (for industrial and lab use) in the lab washer.

Cleaning and Maintenance

The following method describes a routine cleaning between two fermentations/cultivations. It takes place with the culture vessel completely assembled and the accessories completely mounted.

This does not include the sensors, with the exception of antifoam or level sensors from the equipment manufacturer. To avoid damaging the other sensors during the routine cleaning, they are first removed and then cleaned separately according to the third-party manufacturer guidelines and then stored, if necessary. Also see the “Removing Sensors” section and “Cleaning Sensors”.

Proceed as follows to carry out a routine cleaning of the culture vessel:

Procedure

1. Carefully unscrew the sensors (except antifoam/level sensors) by hand (no tools!) from the vessel top plate ports and place them to the side for separate cleaning according to the manufacturer guidelines.
2. Completely fill the culture vessel with 0.1 N NaOH.
3. Fit the top plate on the vessel and secure it.
4. Hang the culture vessel on the basic unit.
5. Couple the motor.
6. Switch on the equipment at the main switch.
7. At the operating panel, start the Batch (process) using **START BATCH** and stir strongly for 2 hours with the stirrer function (parameter *Stirrer*).

INFORMATION

It is recommended to warm the 0.1 N caustic soda to 60 °C and to prolong the duration of stirring for dealing with persistent residue of foam or protein.

8. At the operating panel, stop the Batch (process) using **INOCULATE** and **STOP BATCH**.
9. Switch the equipment off at the main switch.
10. Let the motor cool down.

    When the motor has cooled down:
11. Uncouple the motor.
12. Remove the top plate and carefully place it so that it does not lie on top of components(!)
13. Empty the culture vessel.
14. Thoroughly rinse the culture vessel with distilled water.

11.3 Removing the Vessel Top Plate and Accessories

All accessories must be removed for thorough cleaning of the individual parts of the culture vessel. This is described in the following sections. The cleaning itself is described in the chapter “Cleaning and Storing Individual Parts”.

The cleaning of the hoses with pump heads, the basic unit, operating panel and the exit gas cooler are described in separate sections.

Sensors from third-party manufacturers are cleaned according to their manufacturer’s specifications.

11.3.1 Removing the Exit Gas Cooler

Proceed as follows:

Procedure

1. Unscrew the exit gas cooler from the vessel top plate port by hand.

   Ensure that the O-ring does not get lost.
2. Gently release the hose clamp with the hand wheel, pull off the exit gas filter and dispose of it.
3. Remove the pressure hose piece to thoroughly clean the exit gas cooler. (For details see the chapter “Cleaning the Exit Gas Cooler”.)

11.3.2 Removing the Sensors

Proceed as follows:

pH, pO2

Procedure

1. Carefully unscrew the sensors by hand (no tools!) from the vessel top plate ports.
2. Clean/service the sensors according to the sensor manufacturer guidelines.

Antifoam/level sensor

Procedure

1. Loosen and remove the fastening screw beside the sensor by hand.
2. Loosen the slotted-head screw at the clamping adapter.
3. Carefully remove the sensor from the clamping adapter.
4. Pull the clamping adapter out of the vessel top plate port by hand.

   Ensure that the outer O-ring at the clamping adapter does not get lost and that the insulation is not damaged.

INFORMATION

The sensor can be pulled out of the vessel top plate port along with the clamping adapter. After subsequently unscrewing the slotted-head screw on the clamping adapter, the sensor can be pulled out of the clamping adapter.

11.3.3 Removing Hoses, Filters and Pump Heads

To later clean reagent hoses and pump heads, they must be removed from the reagent bottles and from components of the culture vessel.

INFORMATION

To avoid damage, never dismantle the pump heads. Always replace a damaged pump head along with the pump hose, and vice versa.

Proceed as follows:

Procedure

1. Remove cable ties (e.g. with a side cutter) so that the hoses are not damaged.
2. Pull hoses off the culture vessel and the reagent bottles.
3. Remove and dispose of filters for pressure equalisation and hoses from reagent bottles.
4. Ensure that the inlet air filter is clean, dry and not blocked. If this is not the case, dispose of it.

INFORMATION

If the filter for pressure equalisation and the corresponding hoses have been used several times, ensure that the filters are always dry and clean.

5. Dispose of the exit gas filter (see also chapter “Removing the Exit Gas Cooler”).

11.3.4 Removing Blanking Plugs

Proceed as follows:

Blanking plugs in 10 mm vessel top plate ports

Procedure

1. Loosen and remove the fastening screw beside the blanking plugs by hand.
2. Pull the blanking plug out of the vessel top plate port by hand.

   Ensure that the O-ring at the blanking plug does not get lost.

Blanking plugs in 12 mm/Pg13.5 vessel top plate ports

Procedure

1. Loosen the blanking plug with a hexagon socket spanner and remove it by hand.

   Ensure that the O-ring does not get lost.

11.3.5 Removing the Septum Collar and Septum

Proceed as follows:

Procedure

1. Loosen the blanking plug with a hexagon socket spanner in the septum collar and remove it by hand.

   Ensure that the O-ring does not get lost.
2. Unscrew the septum collar out of the port by hand.
3. Remove the septum from the port and dispose of it.

11.3.6 Removing Addition Port Adapters, Feed Needle and Temperature Sensor Pocket

Proceed as follows:

Procedure

1. Loosen and remove the fastening screw between the addition port adapters and/or feed needle(s) as well as beside the pocket by hand.
2. Pull the addition port adapters and if necessary, the feed needle(s) from the vessel top plate ports by hand.
3. Pull the temperature sensor pocket out of the vessel top plate port.

   Ensure that the O-rings on the addition port adapters and at the pocket do not get lost.

11.3.7 Removing the Vessel Top Plate

Proceed as follows to remove the vessel top plate:

Procedure

1. As far as possible, remove mounted parts before lifting the top plate.
2. Remove the knurled screws on the top plate by hand (no tool!) and place them to the side.
3. Carefully lift the top plate vertically upwards from the vessel until the stirrer shaft and other long components can no longer come into contact with the glass vessel.

ATTENTION

If the vessel top plate presses against long components they could bend because of the weight of the top plate.

Always position the vessel top plate so that it does not lie on top of components.

4. If necessary, now remove components that have not yet been removed.

   Never remove the stirrer shaft!
5. Check the glass vessel for damage (cracks, fissures, scratches) and replace if necessary.

11.3.8 Removing the Sparger and the Dip Tube(s)

Straight spargers and dip tubes can be removed from the outside of the vessel top plate. Curved spargers and dip tubes can only be removed from the inside of the vessel top plate.

Since this equipment uses ring spargers and straight dip tubes, removal from the inside of the vessel top plate is described here. This means that the vessel top plate is already removed.

Proceed as follows:

Procedure

1. Loosen and remove the fastening screw beside the sparger/dip tube by hand.
2. Loosen the slotted-head screw at the clamping adapter.
3. Carefully pull the sparger/dip tube from the bottom out of the clamping adapter.
4. Pull the clamping adapter out of the vessel top plate port by hand.

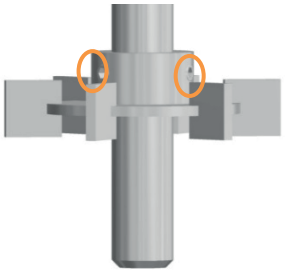
   Ensure that the outer O-ring at the clamping adapter does not get lost.

11.3.9 Removing the Impellers

Before removing the impellers, it is recommended to measure and record the positions to aid later mounting.

Proceed as follows to remove:

Procedure:



1. Loosen the grub screws on the impeller with an Allen key – do not remove!

2. Carefully remove the impeller from the stirrer shaft.

11.4 Cleaning and Storing Individual Parts

The procedure described here applies to the following individual parts:

- Vessel
- Accessories such as blanking plugs, spargers, dip tubes, addition port adapters etc.
- Reagent bottles
- Vessel top plate, with regard to its particular characteristics

Particulars when cleaning the top plate

- Do not place the top plate on the stirrer shaft.
- Never removed the drive hub and stirrer shaft!

INFORMATION

Cleaning of the sensors, hoses and pump heads as well as the basic unit and the exit gas cooler are described in separate sections.

Proceed as follows for cleaning:

Procedure

1. Clean parts with distilled water and a soft sponge or in the dishwasher.

   Ensure that the deposits in the dip tubes and, if necessary, the feed needles, are removed. Use 0.1 N caustic soda solution followed by distilled water as necessary. For this, see the “Cleaning the Culture Vessel” section.
2. Dry all parts, including the inner parts of the dip tubes, spargers and feed needles.
3. Check all O-rings for cracks or damage. Replace them if necessary.
4. Store the vessel, vessel top plate and accessories in a clean, dry state in a location where they cannot be physically damaged (e.g. by falling), or prepare them for the next cultivation.

11.5 Cleaning the Sensors

Apart from antifoam and level sensor, all sensors are cleaned and maintained according to the descriptions of the sensor manufacturer.

Procedure

1. Clean the sensors according to the sensor manufacturer guidelines.
2. Prepare the sensors for the next cultivation or, if necessary, service and/or store them according to the sensor manufacturer guidelines.

11.6 Cleaning the Hoses and Pump Heads

Proceed as follows to clean the reagent hoses and pump heads:

Procedure

1. Thoroughly rinse the hoses with the pump heads with water.
2. Carefully dry all hoses and, if necessary, blow out with compressed air.

INFORMATION

To avoid damage, never dismantle the pump heads. Always replace a damaged pump head along with the pump hose, and vice versa.

11.7 Cleaning the Super Safe Sampler

ATTENTION

Risk of damage to the sampling system from unsuitable cleaning methods or cleaning agent (such as acids, bases or solvents, for example).

– Only use water or a mild soap solution for cleaning.
– The sterile filter must remain dry at all times.

Proceed as follows to clean the sampling system:

Procedure

1. Fill the culture vessel with water or a mild soap solution.

   Or: Remove the sampling hose from the dip tube and hold it in a vessel, e.g. a beaker, with water or a soap solution.
2. Place the syringe on the automatic valve and pull out the plunger to rinse the sampling system.

   When using a soap solution:
3. Then rinse the sampling system thoroughly with water.

INFORMATION

If the test record requires that the culture is killed off after cultivation by autoclaving the culture vessel, the valves of the sampling system may become stuck due to reside of the culture solution. In such a situation, it would be better to autoclave the sampling system separately in a beaker of water (hoses filled with water, filter removed).

11.8 Cleaning the Exit Gas Cooler

If the exit gas cooler is only lightly soiled, an ultrasonic bath for approx. 15 minutes is sufficient to clean it.

However, if foam has entered the exit gas cooler during cultivation, it must be cleaned thoroughly.

To do so, proceed as follows:

Procedure

1. Put the exit gas cooler into 0.1 N NaOH for 4 hours.
2. Rinse the exit gas cooler thoroughly with water.
3. Put the exit gas cooler into an ultrasonic bath for 2 to 5 minutes.

4. Flush the exit gas cooler with ethanol (70%).
5. Thoroughly rinse the exit gas cooler with distilled water.

11.9 Cleaning the Basic Unit and Operating Panel

Proceed as follows to clean the surface of the basic unit and the operating panel as required:

Procedure

1. Switch the equipment off at the main switch.
2. Disconnect the equipment from the mains supply.
3. Wipe all surfaces with a damp cloth.

   Clean with an appropriate disinfectant as necessary.
4. Clean the screen with a wipe suitable for computer or laptop screens.

11.10 Maintenance Plan

WARNING

Non-compliance of this maintenance plan contains a high risk!

It is the responsibility of the user, that this maintenance plan is complied with. Non-compliance will lead to exclusion of liability (see General Terms and Conditions).

The required maintenance for reliable operation is described in the following chapters.

Reduce the maintenance intervals in case increased abrasion is detected during regular checks.

Contact the manufacturer for questions concerning maintenance. For contact details, see page 2.

To be carried out by operator	
Interval	**Maintenance work**
Before each cultivation	Check all hoses and hose lines.
	Check cables for damage and kinks.
	Check that O-rings and gaskets are leak-proof, replace if necessary.
	Check the integrity of all glass parts (vessel, reagent bottles) and replace if necessary.
	Check all filters and replace if necessary. Replace the exit gas filter.
	If necessary, calibrate the sensors.
After every cultivation	Autoclave and clean the culture vessel and accessories.
As required	Clean the basic unit and operating panel.
	Decalcify the equipment.

To be carried out by qualified personnel	
Interval	**Maintenance work**
Every 6 months	Check and calibrate measuring sections (temperature, pH, etc.) with a simulator

11.11 Decalcifying the Equipment

Calcification could block installed parts, lines or valves in the basic unit. It may be necessary to decalcify the equipment if certain faults occur in the temperature control system.

Please note:

- Use phosphoric acid or citric acid (max 5 %) as decalcifier.
- Observe the inlet pressure specified in the “Technical Data” section.
- To warm up the decalcifier and pump it into the basic unit, use a chiller or a water bath and an external pump.
- During decalcification, the decalcifier flows in a circuit between the basic unit and the chiller/water bath.

Proceed as follows:

Procedure

1. Mount the exit gas cooler into the port of the vessel top plate and connect to the basic unit.

Ensure that the valve for the exit gas cooler water supply is open. Open it, if necessary.

2. Hang the culture vessel on the basic unit (hang the vessel holder on hooks on the thermal block).
3. Fill the chiller/water bath with decalcifier.
4. Connect the chiller or water bath to the water inlet and outlet on the basic unit using hoses.
5. To open the corresponding valves in the basic unit, set the temperature on the operating panel to 5 °C (cool).
6. Set the chiller/water bath to 20 °C to 40 °C.
7. Switch on the pump at the chiller/water bath.
8. Let the decalcifier flow through the equipment for an hour.
9. Connect the water inlet hose on the equipment to tap water.
10. Hang the water outlet hose of the equipment at the spout.
11. Rinse the equipment for an hour.

12 Interferences

The following section describes possible reasons for interferences and how to resolve them. Reduce the service intervals in correspondence with the actual loads if interferences become increasingly common. Contact the manufacturer for interferences that cannot be resolved by following the above instructions. For service contact details, see page 2.

12.1 Interferences Basic Unit and Operating Panel

Interference		
Equipment does not work		
Possible cause	**Remedy**	**By**
Power cable not connected.	Connect the power cable.	Operator
Main switch not switched on.	Switch on the main switch.	Operator
Fuses blown.	Check the fuses of the mains connection and the basic unit.	Qualified electrician
Power connection interrupted.	Check the plug and cable at the basic unit and mains socket. Check mains cable for damage and kinks.	Qualified electrician
LED flashes red, Equipment Alarm is shown on the display, power failure during a running Batch (process).	Acknowledge the alarm message, start the batch again if necessary.	Operator
LED flashes red, Equipment Alarm is shown on the display, control system communication is interrupted.	Acknowledge the alarm message, contact the manufacturer’s service department if the fault message returns.	Operator, INFORS HT service technician or specialist dealer
LED flashes red, Equipment Alarm is shown on the display, pressure in the culture vessel is too high.	Acknowledge the alarm message, if necessary replace the exit gas filter or reduce the gas flow rate.	Operator

12.2 Interferences Drive System

Interference		
Motor does not start.		
Possible cause	**Remedy**	**By**
Motor not properly connected.	Check cable connections and connect correctly as necessary.	Operator
The *Stirrer* parameter is not activated.	Activate the *Stirrer* parameter.	Operator
Stirrer parameter setpoint = 0.	Set *Stirrer* parameter setpoint > 0.	Operator
The pO_2 parameter is activated and set to oxygen control via the stirrer (cascade).	Switch cascade off and test operation via the *Stirrer* parameter.	Operator

Interference		
Motor control is volatile, irregular or stops.		
Possible cause	**Remedy**	**By**
The motor cable was plugged out when the basic unit was switched on.	Replace the motor.	INFORS HT service technician or specialist dealer

Interference		
Unusual sounds when the stirrer is switched on.		
Possible cause	**Remedy**	**By**
Stirrer is in contact with other vessel components, e.g. sensors.	Stop the Batch (process). Switch the equipment off at the main switch. Correctly mount the components in the culture vessel.	Operator

12.3 Interferences Temperature Control System

Interferences		
No temperature control.		
Possible cause	**Remedy**	**By**
Parameter *Temperature* is not activated.	Activate the parameter.	Operator
Parameter *Stirrer* is not activated.	Activate the parameter.	Operator

Interference		
No cooling or inadequate cooling.		
Possible cause	**Remedy**	**By**
No water supply or inadequate flow.	Check the water supply and turn the supply tap if necessary.	Operator
Temperature sensor is not inserted.	Insert the temperature sensor into the pocket in the vessel top plate.	Operator

12.4 Interferences Gassing System

Interferences		
No gassing / air bubbles in the culture vessel.		
Possible cause	**Remedy**	**By**
The on-site gas supply has been interrupted.	Stop the Batch (process). Check the on-site gas supply and switch it on, if necessary.	Operator
The *AirFlow* and/or *Gas2Flow* parameter(s) is/are not activated. And/or: Setpoint in the *AirFlow* or/and *Gas2Flow* parameters = 0. Or: The *TotalFlow* = 0 and/or *GasMix* parameter(s) is/are not activated.	Activate the *AirFlow* and/or *Gas2Flow* parameter(s). And/or: Set the setpoint in the *AirFlow* and/or *Gas2Flow* parameters > 0. Or: Set the *TotalFlow* parameter > 0 and activate *GasMix*.	Operator
Hose connection(s) between the basic unit and the culture vessel is/are kinked or clamped.	Check whether the hose connection(s) is/are clamped, if necessary open the clamp(s). Check hose connection(s) for kinks, if necessary route them again or replace them under observation of the sterility requirements.	Operator
Inlet air filter blocked.	Replace the inlet air filter under sterile conditions.	Operator

Exit gas filter blocked.	The overpressure sensor switches gassing off for 10 sec, replace the exit gas filter under sterile conditions.	Operator

Interference		
The desired gas flow rate is not reached.		
Possible cause	**Remedy**	**By**
Blocked holes on the sparger.	Stop the process (Batch), clean the sparger.	Operator

Interference		
Sudden increase in evaporation losses in the culture vessel.		
Possible cause	**Remedy**	**By**
The exit gas cooler does not cool, the *Temperature* parameter is activated.	Check the water supply to the exit gas cooler, restore it if necessary. The exit gas cooler or basic unit is calcified. Decalcify the equipment, if necessary.	Operator
The exit gas cooler does not cool. The control valve for water flow is closed.	Open the control valve.	Operator

12.5 Interferences pH-System

Interference		
No display or incorrect display of pH		
Possible cause	**Remedy**	**By**
Sensor cable not connected or not properly connected.	Connect properly if necessary.	Operator
pH drift during long cultivation.	Recalibrate pH with offline values (“Product Calibration”, see main chapter “Operation”).	Operator
Faulty pH-sensor.	Test calibration with pH 4 and pH 7 buffer. Regenerate or replace the sensor. Consult the documentation of the sensor manufacturer!	Operator

Interferences

Interference		
No pH control.		
Possible cause	**Remedy**	**By**
The *pH* parameter is not activated.	Activate the *pH* parameter.	Operator
Pumps are not switched on.	Switch on pump1 (*Acid*), pump2 (*Base*)	Operator
Incorrect dead band setting.	Check the dead band (Dead Band in PID settings): Switch off or enter a small value.	Operator
No addition of reagents (acids and base).	Check the reagent bottles: Refill if necessary. Check the hose connections between the reagent bottles and the vessel: Connect properly if necessary. Remove clamps if necessary.	Operator
Pump (base/acid) does not operate properly.	Check the pump (acid/base) functionality on the operating panel.	Operator
Pump hose is damaged. Pump does not rotate: Faulty pump head.	Replace pump head.	Operator

Interference		
pH value drifts up and down over time or acid and base are added almost continuously in turn.		
Possible cause	**Remedy**	**By**
Incorrect PID setting in *pH* parameter.	Check the PID settings and adjust as necessary. Change the special proportional factor (*Prop. Term)* or *Dead band* setting.	Operator
Incorrect strength of reagents: Concentration is too weak or too strong.	Check the strength of reagents. Adjust if necessary: 0.1 mol to 2.0 mol.	Operator

12.6 Interferences pO_2 System

Interferences		
No display or incorrect display of pO_2.		
Possible cause	**Remedy**	**By**
Sensor cable not connected or not properly connected.	Check the sensor cable, connect it properly if necessary.	Operator
Faulty pO_2 sensor.	Consult the documentation of the sensor manufacturer. Check the calibration of the pO_2 sensor. Replace the sensor if necessary.	Operator

Interference		
No pO_2 control.		
Possible cause	**Remedy**	**Remedied by**
The *pO_2* parameter and/or cascaded parameter is/are not activated.	Activate parameters.	Operator
The cascade settings are incorrect.	Check the cascade settings and change as necessary.	Operator.
No gas flow into culture vessel.	See faults in the gassing system.	Operator
Fault with control of gas mixing unit.	Check connections. Check gas lines.	Operator

Interference		
Unstable pO_2 control		
Possible cause	**Remedy**	**Remedied by**
Incorrect PID settings in the *pO_2* parameter.	Check the PID settings (*PID* parameter option) and adjust as necessary. Special proportional factor (*Prop. Term*) and dead band. Dead band value must be 0 (zero).	Operator

12.7 Interferences Antifoam/Level Sensor and Antifoam Pumps

Interference		
Foam/medium is not detected.		
Possible cause	**Remedy**	**By**
Sensor is not properly connected.	Check connections and connect properly as necessary.	Operator

Interference		
Foam/medium is always/frequently detected.		
Possible cause	**Remedy**	**By**
Sheathing of antifoam sensor is damaged.	Have the sheathing of the antifoam sensor replaced.	INFORS HT service technician or licensed dealer

Interference		
Antifoam pump does not work.		
Possible cause	**Remedy**	**By**
The *Foam* parameter is not activated.	Activate the *Foam* parameter.	Operator
Pump 3 (*antifoam*) is not switched on.	Switch on pump 3 (*antifoam*).	Operator

Remedy		
No antifoam agent or medium supply or inadequate flow.		
Possible cause	**Remedy**	**Remedied by**
Reagent bottle is empty.	Refill if necessary.	Operator
Incorrect antifoam agent or incorrect concentration.	Replace if necessary.	Operator
Hoses blocked or clamped.	Check the hose connection between the reagent bottle and the culture vessel: If necessary, connect them correctly. Remove clamps if necessary.	Operator
The corresponding pump is not functioning correctly.	Check the function of the pump using the operating panel.	Operator
The pump hose is damaged.	Replace pump head.	Operator
Incorrect hose type connected.	Replace if necessary.	Operator

12.8 Interferences Addition of Feed Solution (Feed Pump)

Interference		
No feed solution or inadequate feed solution.		
Possible cause	**Remedy**	**By**
The *Feed* parameter (pump) is not activated.	Activate the *Feed* parameter (pump).	Operator
Feed parameter (pump) setpoint = 0.	Set *Feed* parameter (pump) setpoint > 0.	Operator
Hose lines blocked or clamped.	Check the hose connection between the reagent bottle and the culture vessel: If necessary, connect them correctly. Remove clamps if necessary.	Operator
Reagent bottle empty.	Refill if necessary.	Operator
Feed pump is not functioning correctly.	Check the function of the feed pump on the operating panel.	Operator
The pump hose is damaged.	Replace pump head.	Operator
Pump head does not rotate: Defective pump head.	Replace pump head.	Operator
Incorrect hose type connected.	Check the hose type. Replace if necessary.	Operator

12.9 Returning for Repair

The provider must return the equipment or the faulty component part(s) to the manufacturer if, after consulting the service department of the local dealer or the manufacturer, on-site diagnosis and/or repair is not possible.

INFORMATION

When returning the equipment, the component part or accessory for repair, it is required for the safety of all parties involved and because of legal provisions that a lawful declaration of decontamination is present. Refer to main chapter “Safety and Responsibility”, chapter “Declaration of Contamination” for details.

13 Disassembly and Disposal

The equipment must be disassembled and disposed of in an environmentally friendly manner if it is no longer in use.

INFORMATION

When returning the equipment for disassembly or disposal, it is required for the safety of all parties involved and because of legal provisions that a lawful declaration of decontamination is present. Refer to main chapter “Safety and Responsibility”, chapter “Declaration of Contamination” for details.

13.1 Disassembly

Prior to disassembly:

- Switch off the equipment and lock any isolation switch in the ‘off’ position.
- Physically disconnect the main energy supply from the equipment and wait for components to fully discharge.
- Remove and dispose of all additional consumable items, auxiliary components and/or spent processing material in an environmentally acceptable manner.

Clean and disassemble component parts professionally with regard to any local regulations concerning employment and environmental protection. If possible, separate materials.

13.2 Disposal

Recycle disassembled components if no agreement is made concerning reclaim or disposal.

- Send metals for scrap.
- Send plastic components for recycling.
- Sort and dispose of the remaining components according their material composition.

Disassembly and Disposal

WARNING

Electronic waste, electronic components, lubricants or other auxiliary materials/supplies are subject to hazardous waste regulations and may only be disposed of by registered specialist disposal firms.

For disposal, the system units are to be disassembled and dismantled into individual material groups. These materials are to be disposed of according to the applicable national and local legislation.

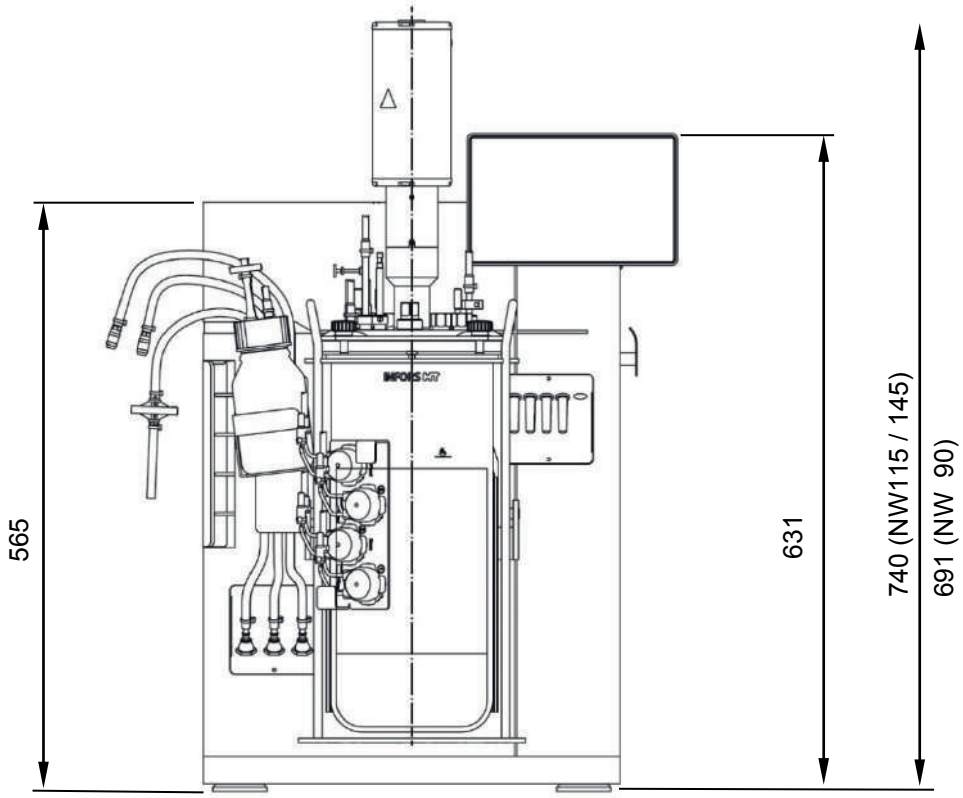
Local authorities or specialist disposal firms can provide information regarding environmentally acceptable disposal.

If no special arrangements have been made for return, INFORS HT units with the required declaration of decontamination can be sent back to the manufacturer for disposal.

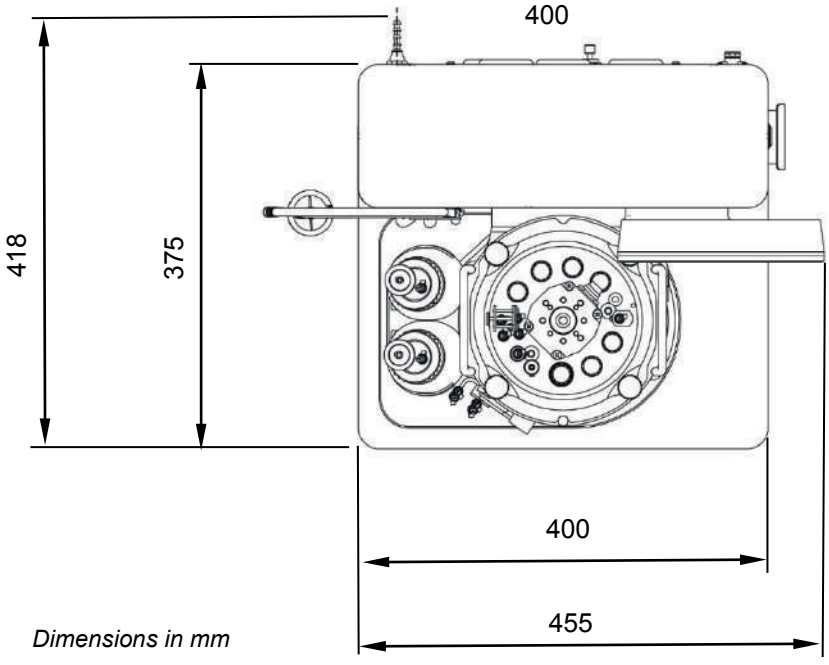
14 Technical Data

14.1 Equipment Dimensions

Front view



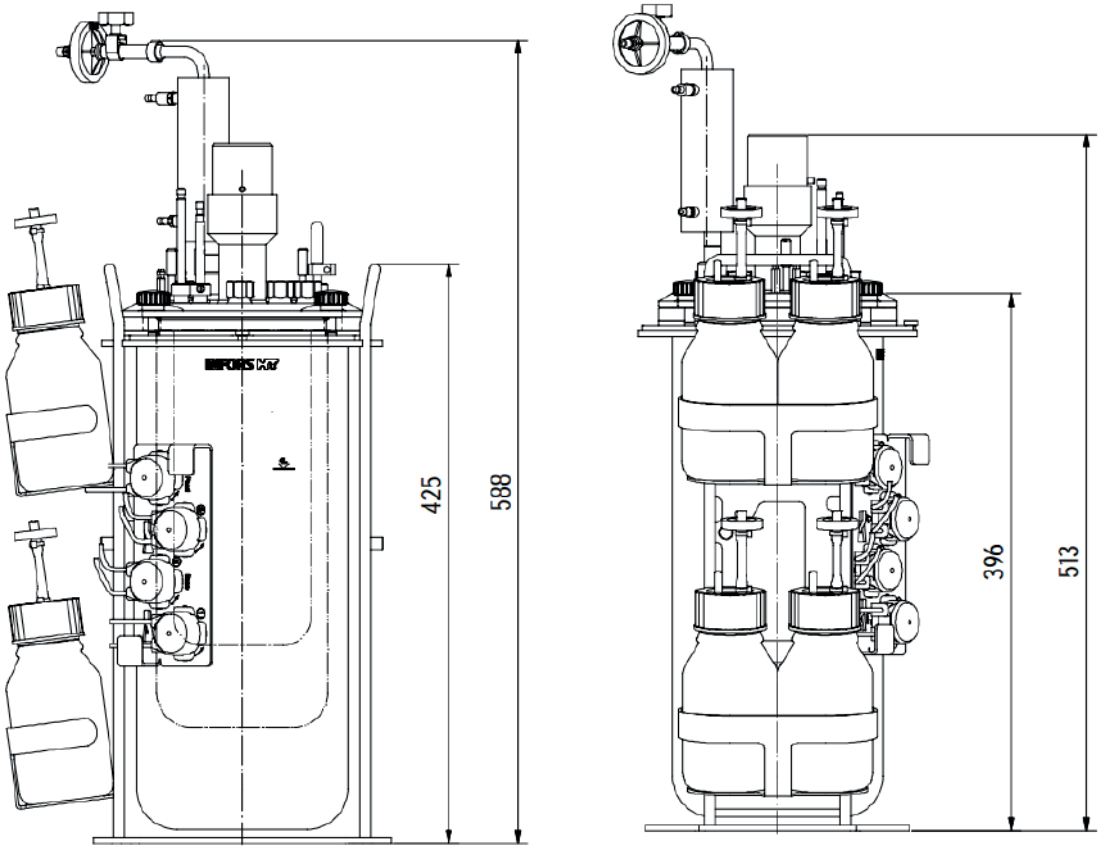
Top view

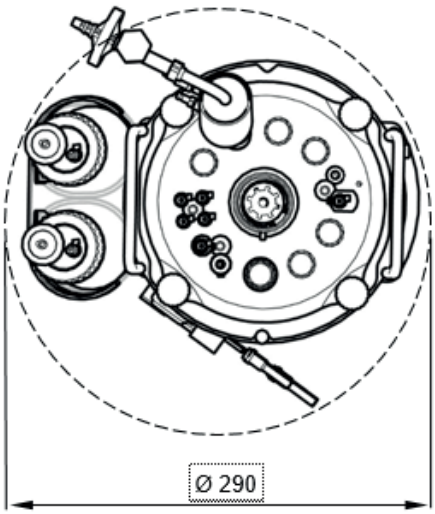


Dimensions in mm

14.2 Dimensions of Culture Vessel

The two dimension drawings show a fully equipped culture vessel ready for autoclaving.





Dimensions in mm

14.3 Weights (netto)

Basic unit	Culture vessel [1]		
	NW 90	NW 115	NW 145
23.5 ± 0.5 kg	6 ± 0.5 kg	7.5 ± 0.5 kg	9.5 ± 0.5 kg

[1]) *Equipped culture vessel, without medium, with vessel holder. The actual weight depends on design and allocation.*

14.4 Connection Requirements

14.4.1 Electrical

Description	Value	Unit
Voltage	120 / 230	VAC
Frequency range	50 / 60	Hz
Max. Current	8	A
Max. power consumption [1]	~ 800	W

[1]) *During heating phase, vessel with max. 4 L working volume, at max. rotation speed.*

14.4.2 Water IN, Basic Unit

Description	Value	Unit
Connection pressure	2 ± 1	bar
Hose nozzle connection, nominal width	6	mm
Water quality	“Very soft” / “soft” ($CaCO_3$ concentration: 0 mmol L^{-1} to 1.5 mmol L^{-1})	

14.4.3 Water OUT, Basic Unit

Description	Value	Unit
Connection pressure	No back pressure	
Hose nozzle connection, nominal width	6	mm

14.4.4 Gas (Air, O_2 or N_2)

Description	Value	Unit
Constant connection pressure	2 ± 0.5	bar
Hose nozzle connection, nominal width	6	mm
General gas quality	Dry, clean and free of oil and dust	
Recommended compressed air quality	Class 1,2,3,4 as per DIN ISO 8573-1	

14.5 Specifications

14.5.1 Operating Panel

Description	Value
HMI	7” colour touch screen
Operating system	Embedded Linux
OPC server	OPC UA
Splash and dust protection	IP 22

14.5.2 Culture Vessels

Vessel

Description	Value
Form	Cylindrical with flat bottom
Operating pressure in culture vessel	Pressureless

Vessel size variants:

TV [1]	Max. AV [2]	Min. AV [3]	NW [4]	Height
1.5 L	1.0 L	0.3 L	90 mm	235 mm
3.0 L	2.0 L	0.6 L	115 mm	295 mm
6.0 L	4.0 L	1.1 L	145 mm	370 mm

1) *Total volume*
2) *Max. working volume*
3) *Min. working volume*
4) *Nominal width = Inner diameter vessel*

INFORMATION

The volume markings on the glass vessels are only intended as visual aids. They do not represent precise measurements in litres.

Ports

Port		Quantity acc. to vessel size		
Ø	**Thread**	**NW 90**	**NW 115**	**NW 145**
7.5 mm	None	4	4	4
10 mm	None	4	4	4
12 mm	Pg13.5	4	6	7

Material

Description	Value
Vessel	Borosilicate glass
Top plate	Stainless steel, AISI 316L, electrolytically polished
Components	Stainless steel, AISI 316L, electrolytically polished
Impellers	**At NW 90:** PEEK **At NW115 and 145:** Stainless steel, AISI 316L, electrolytically polished
O-ring (touches product)	EPDM

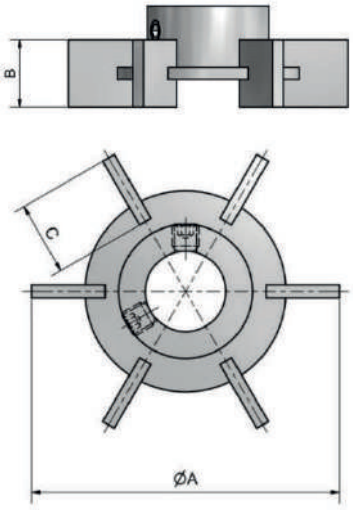
14.5.3 Stirrer

Description	Value
Drive	Shaft with mechanical seal
Motor	Type: DC, brushless
Nominal power of motor	Small motor 102 W Large motor 260 W
Range of rotation speed[1]	**NW 90 (small motor)** 150 min^{-1} to 1,600 min^{-1} **NW 115 (large motor)** 150 min^{-1} to 1,600 min^{-1} **NW 145 (large motor)** 150 min^{-1} to 1,600 min^{-1}

Technical Data

Accuracy	**Measuring:** ± 5 min^{-1} at 100 min^{-1} to 1,600 min^{-1} 1 % setpoint at > 100 % min-1 **Control:** 1 % full scale
Direction of stirrer shaft's rotation	Anti-clockwise = to left
Bearing	Outside vessel in drive hub

1) *Rotation speed is valid for 2 impellers, viscosity similar to water, without gassing.*



Impellers

Quantity / type	Material	
	Vessel NW 145 and NW 115	**Vessel size NW 90**
2 Rushton impellers with 6 blades	Stainless steel, 316L, electrolytically polished	PEEK

Vessel	A	B	C
6 l TV / NW 145	54 mm	11 mm	11 mm
3,0 l TV NW 115	46 mm	11 mm	11 mm
1.5 L TV/NW 90	38 mm	9 mm	11 mm

14.5.4 Temperature

Description	Value
Heating	Electrical, thermal block 630 W
Cooling	Tap water [1] via thermal block and adapter
Sensor	Type: Pt100 1/3 DIN-B
Measuring range	0 °C to +145 °C
Control range	From 5 °C higher than preheating temperature to 60 °C
Accuracy at +20 °C to +60 °C	Measuring: ± 0.1 °C Measuring: ± 0.2 °C

1) *A circulating cooler can be used instead of tap water for the cooling system.*

14.5.5 Gassing

All gas enters via the sparger.

The specific gas flow rate, calculated for the maximum working volume, is 2 min^{-1} for every vessel size.

Variants

Gas(ses)	Controlling flow rate	MFC accuracy
Air	2 MFC [1] (mass flow controller)	± 0.05 slm
Air + O_2		
Air + N_2		

[1]).. 2 are preinstalled even for gassing using air only

Vessel		Control range of gas flow l min^{-1}
TV [2]	Max. AV [3]	
1.5 L	1.0 L	0.05 to 2
3.0 L	2.0 L	0.05 to 4
6.0 L	4.0 L	0.05 to 8

[2]) Total volume

[3]) Working volume

14.5.6 pH

Control system	
Pumps	*Acid* and *base*
Measurement system, digital	
Conventional pH sensor (potential measurement against reference) with built-in electronics	
Type	Easyferm Plus ARC
Manufacturer	HAMILTON
Measuring range	pH 2 to pH 14

INFORMATION

The pH sensors are preconfigured by unit manufacturer INFORS HT. Replacement sensors must be configured before use.

For details on the technical data, usage and maintenance requirements for the pH sensors, see the separate documentation provided by the sensor manufacturer.

14.5.7 pO_2

Control system	
Via cascade	Stirrer Gas flow Gas mixture (addition of O_2 or N_2)
Measurement system, digital	
pO_2 sensor with built-in opto-electronics	
Type	Visiferm DO ARC
Manufacturer	HAMILTON
Measuring range	0.05 % - 300 % air saturation

INFORMATION

The pO_2 sensors are preconfigured by unit manufacturer INFORS HT. Replacement sensors must be configured before use.

For details on the technical data, usage and maintenance requirements for the pO2 sensors, see the separate documentation provided by the sensor manufacturer.

14.5.8 Antifoam

Description	Value
Sensor	Conductive with dosing needle
Control system, digital	Pump 3: *AF* (antifoam)
Range	0 or 100 % (IN or OUT)

14.5.9 Pumps

Type	Quantity	
Peristaltic	4	
Configuration	**Standard**	**Alternative setting**
Pump 1	*ACID,* digital	*FEED,* (analogue)
Pump 2	*Base,* (digital)	*FEED,* (analogue)
Pump 3	*AF* = (antifoam), digital	*LEVEL, (digital)* *or:* *FEED, (analogue)*
Pump 4	*FEED,* (analogue)	*BALANCED, (analogue)*

Hoses	Standard	Option 1	Option 2
Inside diameter	1.0 mm	0.5 mm	2.5 mm
Delivery rate [1]	3.5 ml min^{-1}	1.1 ml min^{-1}	16.1 ml min^{-1}
Material	PharMed BPT		

[1]) *Typical figure with water measured at max. rotation speed*

14.6 Operating Conditions

Description	Value
Temperature range	5 °C up to 40 °C
Relative air humidity, non-condensing	20 % up to 90 %
Min. distance from walls, ceilings and other appliances	150 mm

14.7 Emissions

Description	Value	Units
Noise emission	<70	dB (A)

14.8 Auxiliary Supplies

pH buffers	Intended use
pH 4.0 pH 7.0	For calibrating the pH sensor